विभिन्न नमी नियमों के साथ मिट्टी में फ्लुप्रैडिफ्यूरोन और फ्लुपीरम की दृढ़ता और गतिशीलता

Persistence and Mobility of Flupyradifurone and Fluopyram in Soils with Different Moisture Regimes

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Persistence and Mobility of Flupyradifurone and Fluopyram in Soils with Different Moisture Regimes

By

SUBHASIS SARKAR

A Thesis

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CERTIFICATE

This is to certify that the thesis entitled "**Persistence and Mobility of Flupyradifurone and Fluopyram in soils with Different Moisture Regimes**" submitted to the Faculty of the Post-Graduate School, ICAR- Indian Agricultural Research Institute, New Delhi, in partial fulfilment of the requirements for the award of the degree of **Master of Science** in **Agricultural Chemicals** embodies the results of bona-fide research work carried out by **Mr. Subhasis Sarkar** under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by him.

Irani Mukherjee

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Place: New Delhi, India

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Subhasis Sarkar
SUBHASIS SARKAR

A Blessing from

The Almighty…

…An offering to my

Parents and my Chairperson

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Chapter 1

INTRODUCTION

With a significant growth record, agricultural production in India has grown tremendously over the previous few decades. Overall food grain production achieved 277.49 million tonnes during 2017-18. In addition, the complete food grain production in the nation is reported at 281.37 million tonnes during 2018-19 (including 2018 Kharif and 2018-19 Rabi crops), according to 2nd advance estimate. This is over 4 million tonnes greater than last year's output. Rice production is estimated at 115.6 million tonnes, 4.59 million tonnes greater than 111.01 million tonnes of preceding production in 2017-18. In 2018-19, wheat output is estimated at 99.12 million tonnes compared to the past year's 97.11 million tonnes (http://indiabudget.nic.in).

The primary challenge facing modern farming is how to enhance food production quality and quantity while keeping away any destructive impact on environmental or natural resources. The use of agrochemicals to eradicate pests and disease is one of the main components in sustainable agriculture. As in simultaneous, the environmental impact of these chemicals is of major concern. It is important that agrochemicals exist in the field throughout the cultivation cycle for the required time period to control pests. Sensitive management of these inputs, however, was quite essential for precision farming, where use of agrochemicals continually increases toxicity in food, feed and the atmosphere. As long as the time of persistence of a molecule prolonged in the biosphere, a consequent risk to the sensitive individuals will be higher.

The main temporary reservoir for the accumulation of pesticide residues is soil. Adsorption, migration and degradation are the main procedures responsible for the behaviour and fate of a pesticide when it appears in the soil (Gavrilescu, 2005; Navarro and Kah, 2007). In water and soil, the persistence of residues of hazardous pesticides harmfully affect quality of soil, aquatic organism, and groundwater. Use of pesticides for the purpose of irrigation results in pollution of groundwater, leads to a contamination of food chain. The persistence and downward mobility of freshly introduced pesticides in the soil must therefore be studied (Rao and Davidson, 1980).

At present, the most important insecticides used in the world are Neonicotinoids because of their advantageous characteristics such as low rate of application, flexible

implementation techniques, a wide range of activity and systemic upward mobility in crops (Goulson, 2013; Bass *et al*., 2015). They have been in use since the early 1990s, the first compound commercially introduced in 1991 being imidacloprid. Other insecticides of this class were successively manufactured from 1995 to 2000, including acetamiprid, thiamethoxam, and thiacloprid. All these insecticides are agonists to nicotinic acetylcholine receptors (nAChRs) in the insect's central nervous system (Navarro *et al*., 2009). However, in the nectar and pollen of treated plants, their presence has also been found, which in turn can lead to honeybee exposure (Fairbrother *et al*., 2014). Consequently, the European Food Safety Authority (EFSA, 2019) following a re-evaluation of the hazards induced by the neonicotinoids and limited the use of certain neonicotinoid insecticides (imidacloprid, clothianidin and thiamethoxam) in plants with adverse effects on bees and other pollinators (European Commission, 2013). In addition, neonicotinoids can persist and dissipate in soils and leach down through the soil profile and cause of pollution of the ground water (Goulson, 2013; Raina-Fulton, 2016).

Flupyradifurone, 2(5H -(furanone), 4-{[(6-chloro-3-pyridinyl)methyl] (2,2 difluroethyl) amino} is a novel compound, proposed for apply as a systemic insecticide, designed by Bayer CropScience under the trade name Sivanto^{TM}, licensed and marketed worldwide in all main climatic areas, the main markets being Brazil, the United States, Europe, Ghana, India and China (Starner and Goh, 2012). The chemical is a member of the butenolide class of insecticides and insecticidal activity of flupyradifurone is similar to neonicotinoids. The chemical is an agonist of the receptor, nicotinic acetylcholine (Sun *et al*., 2018). Due to this mode of action, the compound has been placed in the Insecticide Resistance Action Committee (IRAC) Group 4 class of insecticides (IRAC, 2012).

Flupyradifurone engaged in excitatory neurotransmission, interacts with insect nicotinic acetylcholine receptors. The nicotinic acetylcholine receptor is similarly the site of target for neonicotinoid insecticides. As the compound functioning as an agonist, depolarizing ion current has been generated when flupyradifurone binds to the receptor protein followed by nerve cell excitation. In difference to, natural acetylcholine, Acetylcholinesterase cannot activate Flupyradifurone, leading into an insect's nervous system disturbance and subsequent insect death.

Foliage application of flupyradifurone is permitted in plant group 15 cereal grains (except rice), seed root vegetables (except sugar beet), legume vegetables (succulent or dried), cotton, tuberous and corm vegetables, non-grass animal feed, leafy vegetables (except Brassica), and soybean seeds (United States Environmental Protection Agency, Washington DC, June25'2014). For a wide range of pests such as aphids, hoppers and whiteflies this compound displays an excellent biological effectiveness in small quantities. Extension of the spectrum to weevils of soft scales, mealybugs, flea beetles, psyllids and leafminer is being examined. Foliage application of Sivanto^{TM} is most efficient as a threshold treatment and provides excellent crop coverage.

The nature of flupyradifurone is persistent to very persistent and moderate to less mobility has been observed for the compound, but it varies according to soil conditions. So, the compound has the ability to outreach aquatic ecosystem, including groundwater, for various months or longer after implementation (Chawla *et al*.,2018). It is non-volatile and therefore air movement is not going to be a significant transportation route. The accessible fate data indicate that dissipation of flupyradifurone from the point of application is taken place through multiple transport processes, including leaching to groundwater, erosion and runoff, although time to 90% decrease in mass of pesticide (DT_{90}) from surface soil often surpassed one year in terrestrial field dissipation research (Ju *et al*., 2015). Based on accessible degradation information, the concerned residues for aquatic exposure estimation are parent Flupyradifurone and uncharacterized metabolites (Gulkowska *et al*., 2014). Flupyradifurone soil sorption is affected by the percentage of organic carbon, suggesting potential for movement with runoff and/or infiltration of soil and leach into groundwater. The results of aquatic field dissipation information indicate that most residues of flupyradifurone may not be moved quickly into sediment pore water; however, there may be inconsistent residues in sediment. Values of K_{oc} suggest that flupyradifurone has a greater organic matter empathy than water (Wei *et al*., 2016). A higher proportion of flupyradifurone is anticipated to be present in the water column relative to sediment in most aquatic habitats. Flupyradifurone has the ability to move through the soil into groundwater and into aquatic environments through surface run-off, it can impact some species of aquatic invertebrates from the soil and foliar applications, useful arthropods and foliar

applications, and may pose a danger to birds and small wild mammals when used to treat soybean seed.

According to The Central Insecticide Board and Registration Committee (CIB & RC) [Under the Ministry of Agriculture and Farmers Welfare, Govt. of India], Approved source of Import for Flupyradifurone is Bayer CropScience AG, Germany. The compound has no Indigenous Manufacturer. In India, the registered formulation for the compound is 17.09% w/w SL and it is recommended for the use in Okra to control whitefly and jassids @ 250g a.i/ha.

"Fluopyram (N-{2-[3-chloro-5-(trifluoromethyl)-2-pyridyl] ethyl}-α, α, α-trifluoro-o-toluamide), a compound of pyridylethylamide". It has been recorded as a fungicide with a broad range of activity designed for use in many horticultural and arable plants (strawberries, peas, apples, outdoor and indoor lettuces, pear, peppers and beans) to protect against a range of Deuteromycetes and ascomycetes (Chawla *et al*., 2018). It was invented by Bayer CropScience and introduced under the trade name Luna Privilege in April 2012. In the mitochondrial respiratory chain, Fluopyram functioning as an inhibitor of succinate dehydrogenase (complex II), thus transport of electrons has been blocked (Veloukas *et al*., 2012). Functions of Fluopyram comprises of inhibition of fungal spore germination, germ tube elongation, sporulation and mycelium development. It is translaminar in plants and shows some motion in xylem.

Few studies have been performed in order to explain the pattern of dissipation of the compound and its hazard evaluation, when used as a foliage applied fungicide (Chawla *et al*., 2018). In soil, Fluopyram residues were standard. "Residues of its metabolite and benzamide were not recognized in soil after 15 days of usage". Because Fluopyram has a very low vapor pressure of 3.10×10^{-06} Pascal at 25 °C, more soil residues were generated due to low vapour pressure (Gulkowska, *et al*., 2014, Anastassiades *et al*., 2017). In addition, soil drenching also leads to elevated residue levels in soil. Higher levels of fluopyram may result in rapid fluopyram mobility in soil posing a danger to the environment and other soil organisms. K_d and K_{oc} measure the mobility of a substance in soil. K_{oc} is the soil organic carbon, water partitioning coefficient and is the ratio of a chemical adsorbed in the soil by unit mass of organic carbon in the soil by equilibrium chemical concentration in solution, and K_d is the coefficient of distribution between the two phases (notes on chemical parameters, physical and chemical properties). A greater value implies that the material is heavily

adsorbed into soil and organic matter and does not pass through the soil. Further studies of Fluopyram translocations in the soil can be performed to better understand environmental hazards, as shown at K_{oc} 266–460 in soil, suggests mild Fluopyram mobility in soil.

According to The Central Insecticide Board and Registration Committee (CIB & RC) [Under the Ministry of Agriculture and Farmers Welfare, Govt. of India], Approved source of Import for Fluopyram is Bayer CropScience AG, Germany. The compound has no Indigenous Manufacturer. In India, the registered formulation for the compound is 34.48% w/w SC and it is recommended for the use in Tomato as a nematicide to control Root Knot Nematode (*Meloidogyne incognita*) @ 250 gm active ingredient/ha (for two times application) and 500 gm active ingredient/ha (for single application).

The literature demonstrates data gaps concerning flupyradifurone and fluopyram persistence and mobility in various types of soil in the subtropical region. Keeping this background information in mind a study titled: **"Persistence and Mobility of Flupyradifurone and Fluopyram in Soils with different moisture regimes"** is planned with the subsequent objectives:

1. Flupyradifurone and Fluopyram persistence study in different type of soils and in FYM with different moisture regimes.
2. Flupyradifurone and Fluopyram's mobility behaviour study in different types of soils.

Chapter 2

REVIEW OF LITERATURE

2.1 General information

Systemic neonicotinoids (a class of neuroactive insecticides chemically analogous to nicotine) are used to safeguard a broad range of plants, including vegetables, pome and stone fruits, citrus, rice, cotton, maize, potato, sugar beet, oilseed and soybean (Simon-Delso *et al*.,2015). Since their discovery in the early 1980s, neonicotinoid pesticides have become the most extensively used class of insecticides globally, based on their effectiveness in controlling many insect pests and their systemic activity. Neonicotinoids account for one quarter of the global insecticide market by 2008 (Jeschke *et al*., 2010), and this level is rising. (Van der Sluijs *et al*., 2013).

As a member of the new class of butenolide insecticides, flupyradifurone (4-[2,2-difluoroethyl)amino]-2(5H)-furanone) contains a novel bioactive scaffold as a pharmacophore (Jeschke *et al*., 2015), invented and manufactured by Bayer CropScience. It is very flexible in terms of techniques of application to a variety of plants and as a seed treatment product for wide acre plants, displays great and rapid action against a wide spectrum of sucking pest insects including targeted resistant neonicotinoid pest communities such as whiteflies and aphids expressing mechanisms of metabolic resistance. Flupyradifurone interacts with postsynaptic nicotinic receptors of acetylcholine (nAChRs) as a partial agonist. Despite it having the same mode of action as neonicotinoids, it is shown to be efficient against those pest species which, due to their distinct chemistry, show resistance to commercially accessible insecticides of this chemical class. For the control of damson hop aphid, whiteflies and jassids, flupyradifurone proved to be a fresh resistant management tool and against such chemicals to other group of insecticides, it does not show any important cross resistance. Low acute toxicity has been found for flupyradifurone (BYI 02960). In rats, the LD_{50} was equivalent to 2000 mg / kg body weight, at this dose point, 4 to 6 deaths were noted. Flupyradifurone neonicotinoid is mainly used as foliar applied insecticides, however, soil treatment and seed soaking can also be done. And

Flupyradifurone functioning as an acute contact and stomach poison, combining systemic features with relatively small levels of implementation (https://docplayer.net).

Fluopyram (*N*-{2-[3-chloro-5-(trifluoromethyl)-2-pyridyl]ethyl}-*α, α, α*-trifluoro-*o*-toluamide) is a latest broad-spectrum fungicide under "the subgroup of pyridinyl ethylbenzamides, a group of chemicals within the class of SDHIs". Against all the developmental stages of the fungus, starts from the spore development to spore germination, the compound is biologically active, and its range of action comprises of numerous pathogens belonging to ascomycetes & deuteromycetes, such as *Monilinia* spp., *Sclerotinia* spp. and *Botrytis* spp., on crops like vegetables, pome and stone fruit (Labourdette *et al*., 2010). Fluopyram is a long-chain, extremely versatile molecule that can help fit wild and mutated binding sites better (Luna Fungicide., Product Boucher, Bayer 2017). Luna® is a family of three strong new fungicides designed by Bayer CropScience based on the active ingredient fluopyram. Fluopyram is non-volatile, UV-stable and has a low water solubility that improves rainfast properties. Furthermore, it continues to penetrate closed bud, stem, leaf and fruit surfaces to protect from within. This continuous uptake through stems and leaves improves disease control by giving long-term protection to unsprayed tissues. Fluopyram disrupts the citric acid cycle by inhibiting the succinate hydrogenase, at complex II of the fungi's respiratory chain. In simple terms it blocks electron transfer in the mitochondria (Bonmatin *et al*., 2015). Because it binds at this site, fluopyram is unlikely to develop cross-resistance with strobilurin chemistry which binds at complex III of the respiration chain (Technical Guide, Luna Sensation 2017). Fluopyram is formulated as a 500 g ai/L suspension concentrate (SC),containing 41.5% or 4.16 lbs fluopyram/US gallon. Combination products with 50:50 mixtures of fluopyram and prothioconazole (200+200 SC), tebuconazole (200+200 SC) or trifloxystrobin (250 + 250 SC) (Katna *et al*., 2018 and Podbielska *et al*., 2017) and a 1:3 mixture with prothioconazole (125 + 375 SC) have also been formulated (Joint, F.A.O, World Health Organization, & WHO Expert Committee on Food Additives, 2017).

2.2 Outline Description of Flupyradifurone & Fluopyram:

Table 1A: Outline Description of Flupyradifurone

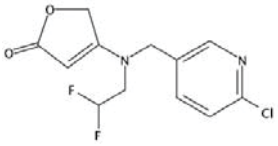
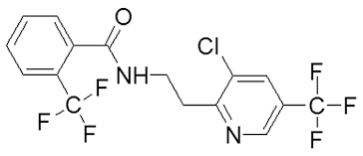
IUPAC Name	4-{[(6-Chloropyridine-3-yl)methyl] (2,2-difluoroethyl)amino}furan-2 (5*H*)-one
Chemical Formula	$C_{12}H_{11}ClF_2N_2O_2$
Weight of the Molecule	288.68 $g.mol^{-1}$
Physical state & Color	Solid (powder) & White to beige
Odor	no characteristic odour (pure Active ingredient)
Melting Point	69° C (pure active ingredient))
Bulk Density	D =1.43 (purity%: 99.4)
Vapor Pressure	9.10 x 10^{-07} Pascal (at 20° C)
Solubility (at 20° C)	✓ In water: 3.2 g/L (pH 4, 20° C) ✓ in organic solvents (g/L): ▪ n-heptane - 0.0005 ▪ toluene - 3.7 ▪ 1,2-dichloromethane - > 250 ▪ ethyl acetate - > 250 ▪ methanol - > 250
Partition Co-efficient	log P = 1.2 (at 25°C and pH 7)
Boiling Point	no boiling point at atmospheric conditions, pure active substance decomposed at 270° C
Trade Name	Sivanto™ (Bayer CropScience)
Type of Formulation	SL (Soluble Liquid), FS (Flowable Concentrate for seed treatment)
Mode of Action	Partial Agonist of nicotinic Acetyl Choline Receptor (nAChR)
Mammalian Acute Oral LD_{50} ($mg.kg^{-1}$)	<2000 (rat)
Structure	

Table 1B: Profile of Fluopyram

IUPAC Name	*N*-{2-[3-chloro-5-(trifluoromethyl)-2-pyridyl]ethyl}-α,α,α-trifluoro-*o*-toluamide
Chemical Formula	$C_{16}H_{11}ClF_6N_2O$
Weight of the Molecule	396.72 g/mol
Physical state & Color	White powder
Odor	No noticeable odour
Melting Point	118 °C at 1013 hPa.
Bulk Density	1.53 at 20 °C
Vapor Pressure	1.2×10^{-06} Pascal for 20 °C
Solubility (at 20° C)	• in water: 15 mg/L (at pH 4 and 20° C) • in organic solvents (g/L): ▪ n-heptane –0.66 ▪ toluene –62.2 ▪ 1,2-dichloromethane - > 250 ▪ ethyl acetate - > 250 ▪ methanol - > 250
n-Octanol/waterPartition Co-efficient	log P_{ow} 3.3 at pH 6.5
Boiling Point	319 °C.
Temperature of decomposition	decomposition range 300–395 °C
Trade Name	Luna® (Bayer CropScience)
Type of Formulation	Suspension Concentrate (SC)
Mode of Action	Succinate dehydrogenase Inhibitor
Acute Oral Toxicity	LD_{50} (rat) 2,000 mg/kg
Structure	

2.3 Synthesis

2.3.A. Synthesis of Flupyradifurone

To discover the new chemical scaffolds for the control of sucking pest, "isolation of natural product stemfoline **1** (Fig. 1), was done from the leaves and stem of the oriental medicinal plant *Stemona japonica* (Blume) [Family-Stemonaceae] and considered as a potent agonist of insect nicotinic acetylcholine receptor (nAChRs), known as a good starting point and broadly used as a potent lead structure in order to identify novel active ingredients for modern crop protection" (Jeschke *et al*.,2013). At present, based on the stemfoline cage structure, designing of small compound is going on. The pyridinyl-cyanotropanes class has been discovered according to this cage structure, for instance the *in vivo* highly active 3-(5-chloro-3-pyridinyl)-8-(2,2,2-trifluoroethyl)-8-azabicyclo[3.2.1]octane-3-carbonitrile **2a** (R=CH_2CF_3) (Fig. 1), which is bioactivated in larval *Heliothis virescens* (Fabricius) by cleavage of the *N*-(2,2,2-trifluoroethyl) residue **2b** (R=H) (Fig. 1) (Urch *et al*.,1999 and Lind *et al*.,2002). While studying the impact of the stemfoline head group on the efficacy of **1** the five ring butenolide became the interest of researchers as a starting point for the preparation of new bioactive scaffolds and finally the identification of the biologically active enaminocarbonyl compound class (**I)**(Fig. 1). The butenolide lead structures **VI** (R_1=CH_2CHR-F) (Fig.-2) (in all structures of subclasses **IV** to **VIII**, changes are highlighted by frames.) were identified by stepwise chemical optimisation of enaminocarbonyl compounds via forming different active butenolide subclasses like **IV** (Z=O) and **V** (A=Het). Figure 4 shows general synthetic pathways to butenolide **VII** via tetronic acid **5** or five-ring lactone intermediates **X** by two different **methods A and B** (Jeschke et al.,2012). Starting with **5**, the butenolides **VII** can be prepared either by treatment with 2-fluoro-containingethylamine(R=H, F) following *N*-alkylation of the intermediates **X** with 2-chloro-5-chloromethylpyridine (**method A**) or by coupling of **5** with secondary amines of type **XI** (**method B**).

By comparing with already commercialised nAChRs agonists such as *N*-cyanoamidines (acetamiprid, thiacloprid), nitroenamines(nitenpyram), *N*-nitroguanidines (imidacloprid, clothianidin, thiamethoxam or dinotefuran) or sulfoximines (sulfoxaflor), It was considered that the butenolide flupyradifurone **4**(Z=O) contains a different pharmacophore system as a new bioactive scaffold and for further global development Bayer CropScience select it under the trade name Sivanto^{TM}[SL 200 g.L^{-1}] (Jeschke *et al*., 2015).

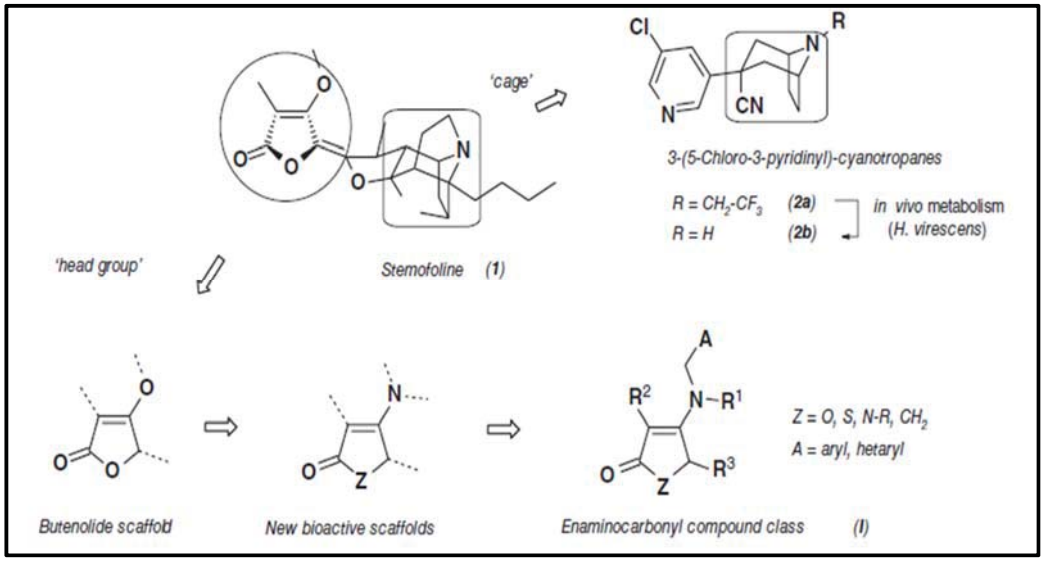


Fig.1. Natural product stemfoline**1** as the lead structure for novel ligands, e.g. **2a, 2b, I**

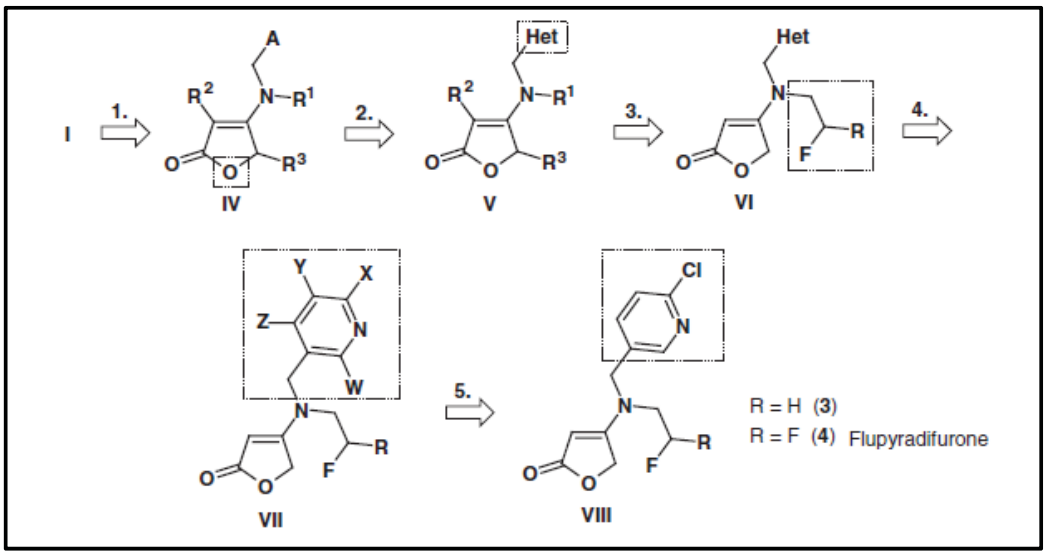


Fig.2. Stepwise chemical evolution of enaminocarbonyl compound **I** via the active butenolide subclasses **IV** to **VIII,** resulting in the discovery of Flupyradifurone **4**

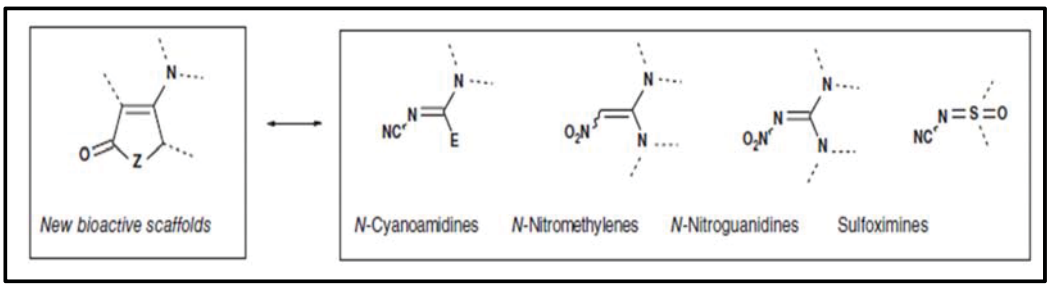


Fig.3. New bioactive scaffold versus pharmacophore systems of known nAChR agonist

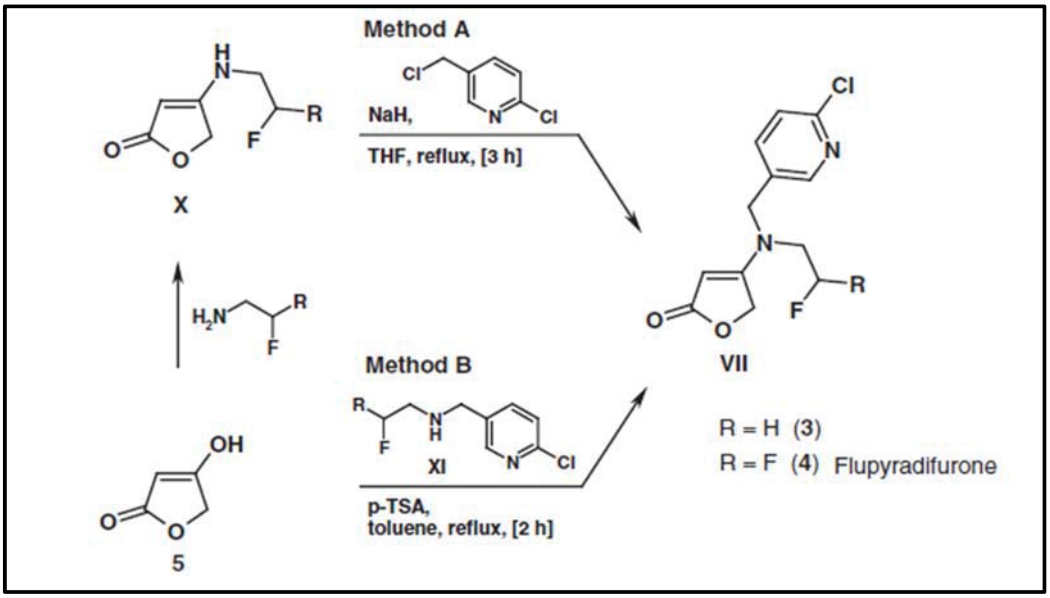


Fig.4. General synthetic pathways to butenolides **VII** by **methods A and B**

2.3.A. Synthesis of Fluopyram

In order to discover novel environmentally friendly agrochemicals with novel structures and mechanisms, introduction of active groups of natural products is an effective method. 1,2,3,4-tetrahydroquinoline (THQ) derivatives, widely existing in natural products (Saha *et al*., 2016), have been studied extensively because of their good bioactivities such as fungal chitin synthase inhibitory activity. Isolation of a novel alkaloid consisting of two THQs structures, aspernigerin (Fig. 1) has been done from extract of culture of *Aspergillus niger* in 2006, considered to be a potential lead compound. Benzamide moiety is an important functional group in both medicinal chemistry and agrochemicals. Interest of researchers have been developed in this field because of their excellent bioactivities until today (Huang *et al*., 2013). For instance, a series of 2-(acetoxy)-benzamide derivatives (Fig. 2), reported by Xiaorong Tang in 2014, exhibited good antifungal activities. In search of a new lead compound having high biological activity, researchers introduced THQ into the benzamide moiety, designed and synthesized a series of N-substituted benzoyl-1,2,3,4-tetrahydroquinolyl-1-carboxamide compounds, and design idea is shown in Scheme-1. The target compounds were synthesized from substituted benzoic acid in four steps (Scheme-2) (Wei *et al*., 2016)

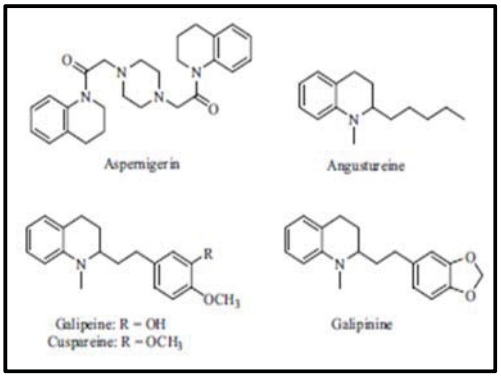


Fig.1. Structures of natural compounds

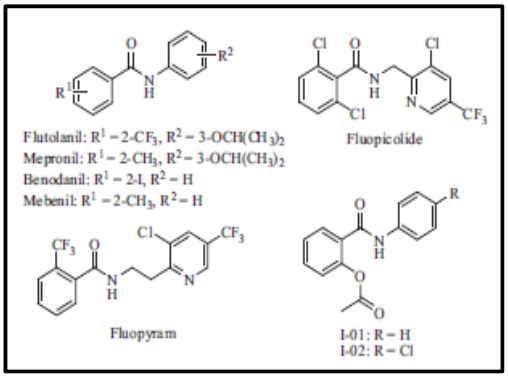
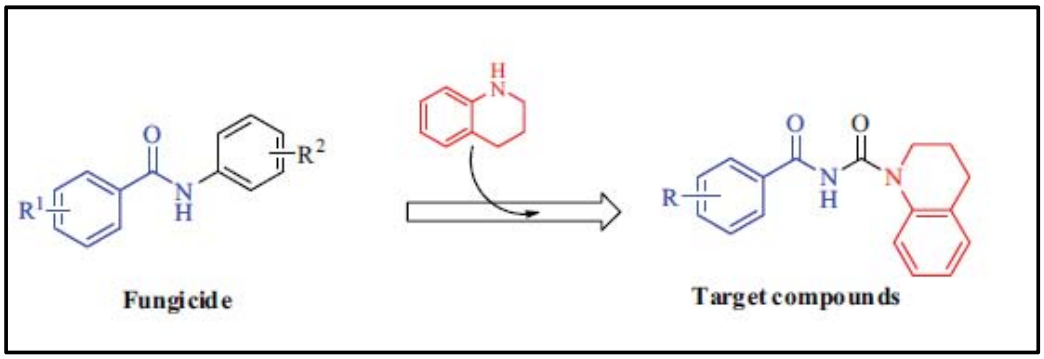
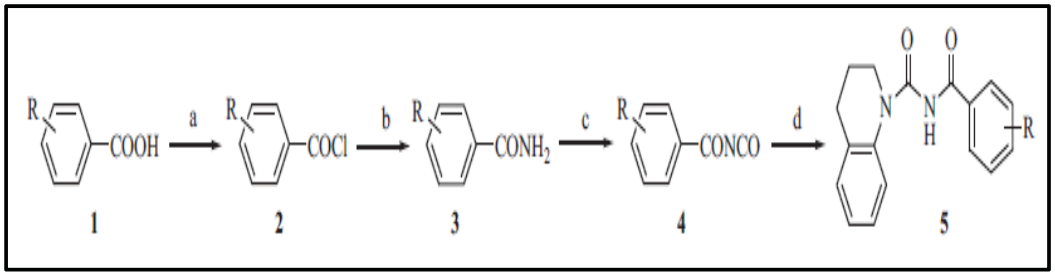


Fig.2. Compounds containing benzamide



Scheme-1: The design idea of target compound



Scheme-2: Synthetic route of the target compounds. Reagents and conditions: (a) $SOCl_2$, toluene, reflux, 3 h; (b) aqua ammoniae, 0–10 _C, 1 h; (c) $(COCl)_2$, 1,2-dichloroethane,reflux, 6 h; (d) 1,2,3,4-tetrahydroquinoline, toluene, reflux, 3 h.

2.4 Analytical methods for estimation of Flupyradifurone and Fluopyram

2.4.A Analytical methods for estimation of Flupyradifurone

An efficient analytical method for successively evaluating a new insecticide namely, flupyradifurone and 2 metabolites of the parent compound was constructed using UHPLC with tandem mass spectrometry combined with a QuEChERS technique. Octadecylsilane and acetonitrile was used for the clean-up and extraction

of the three target compounds followed by effective separation among 3.1 and 1.9 minute using a chromatographic HSS T3 column linked to a source of electrospray ionization. An acceptable recovery of 116.5-70.3 per cent with RSD (relative standard deviations) below 18.6 per cent has been achieved at three spiking levels (0.5, 0.05, and 0.01 ppm) for all the matrix-matched sample. The quantity limits (LOQ) for flupyradifurone and difluoroethylamino-furanone were 10 µ/kg and for 6-chloronicotinic acid were 100 µ/kg. The flupyradifurone quantification limit was well below the maximum residue limit in the United States (Zhang *et al*., 2016).

Neonicotinoid insecticides and the closely associated sulfoximines and butenolides insecticide classes have lately drawn increasing concerns about their potential adverse impacts on non-target species, including pollinators such as bees. Certainly, it becomes progressively apparent that these impacts can happen at significantly reduced concentrations than those deemed safe for animals. Here, scientists used honey as a credible environmental sampler and created an inconceivably delicate application based on Quick Easy Cheap Effective Rugged and Safe procedure and Ultra High Performance Liquid Chromatography coupled with tandem Mass Spectrometry to ascertain the 9 neonicotinoid insecticides and relevant molecules existing in market concurrently such as "acetamiprid, clothianidin, dinotefuran, flupyradifurone, imidacloprid, nitenpyram, sulfoxaflor, thiacloprid and thiamethoxam". The technique was validated and offered exceptional accuracy and precision in the series of 3-4 orders of magnitude. Depending on the analytes, lowest quantification limits (LOQs) were accomplished as low as 2-20 pg / g of honey. The technique was then used to analyse 36 samples of honey from various part of the Globe. They noticed that the five pesticides remained relatively stable at -20 °C for several years, but at Rt (Room temperature), partial degradation was observed in case of acetamiprid and thiacloprid. They also evaluated dinotefuran, nitenpyram, sulfoxaflor and flupyradifurone concentrations and discovered that at least one of these pesticides contaminated 28% of the specimens (Kammoun *et al*., 2019).

2.4.B. Analytical methods for estimation of Fluopyram

Monoclonal antibody-based methods in the agro-food industry have become a helpful analytical technology. In the programs of quality control, fluopyram residues were noted at an increasing rate. In this research, preparation of novel derivatives of this pesticide has been offered and for the 1st time designing of specific and high-

affinity monoclonal antibodies of fluopyram has been done. In addition, "immunoassays to fluopyram were constructed using homologous and heterologous assay conjugates in two complementary enzyme-linked immunosorbent assay formats with detection limits below 0.05 µg / L". The immunoassay optimized for this research had also been used for the investigation of fluopyram in spiked prunes and four distinct types of grape as well as in in-house prepared musts and wines. Retrievals ranged from 109.6 per cent to 76.3 per cent and below 20% were the coefficients of variation. The peak residue limits were well below the quantification boundaries. The achievement of the immunoassay was validated statistically using a chromatographic reference method using samples from plum treated with fluopyram and grape cultivar (Ceballos-Alcantarilla, *et al*., 2019).

A technique for determination of fluopyram and tebuconazole in onion on Liquid Chromatography coupled with tandem Mass spectrometry using dispersive Quick Easy Cheap Effective Rugged and Safe procedure has been validated. 3 sprays of the fluopyram [Trade Name-Luna experiment (400 SC)] + tebuconazole combination fungicide has been applied at an interval of 10 days with Knapsack sprayer @ 75 + 75 and 150 + 150 gm active ingredient/ha. In the bulb setting phase, the first spray was delivered. The samples of spring onions at 20, 15, 10, 7, 5, 3, 1 and 0 (1h) days were taken and the onion bulbs matured after the last spring spray were taken (52 days). Samples of soil have also been collected during harvest. The combined product of foliar application led in 2.86 and 1.14 ppm residues of fluopyram on spring onion at double dose and single dose, one hour after the last implementation respectively. At 20^{th} days of application with half-lives of 9.1 and 8.8 days (at double dose and single application) concentrations of fluopyram residues decreased gradually and reported to be as 0.25 and 0.58 $mg.kg^{-1}$, respectively. For tebuconazole, 0.92 and 2.29 mg kg^{-1} were the respective residues observed after 1 h (0 day) implementation. With half-lives of 7.7 to 6.7 days at double dose and standard dose respectively, the levels declined gradually to 0.33 and 0.12 ppm, respectively, on the 20th days. Here, for fluopyram and tebuconazole at spring onion when applied at a rate of 75 + 75 g a.i ha-1 (400 SC), a 7-day pre harvest interval is proposed by the researchers. (Chawla *et al*., 2018).

For the concurrent identification of fluopyram and tebuconazole in watermelon and soil, a delicate and selective technique has been created. The procedure encompasses a high speed blender extraction process of acetonitrile, a scatterable solid-phase (d-SPE) clean-up procedure and a gas chromatography spectrometry determination (GC-MS) procedure. For watermelons and soil samples of 0.01, 0.1 and 0.5 mg / kg, this technique was validated. Fluopyram and tebuconazole were retrieved on average within such a range of 88.2–106% with RSDs spanning from 1.5% to 7.65%. For both compounds a 0.01 mg / kg quantification threshold (LOQ) was achieved (Dong and Hu., 2014).

2.5 Bioefficacy of Flupyradifurone & Fluopyram

2.5.A. Bioefficacy of Flupyradifurone

A greenhouse study was conducted by using RBD (Randomized block design) for the evaluation of effects of six insecticides on the spread of Tomato yellow leaf curl virus (TYLCV) by the silverleaf whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) to tomato, *Lycopersicon esculentum* (Solanaceae), seedlings that were inoculated with whiteflies from a TYLCV colony in cages 3, 7, or 14 d after treatment with insecticides. The cause of this experiment was to find out the dissimilarity in residual efficacy of four materials that are nearing registration for use on tomato—cyazypyr, flupyradifurone, pyrafluquinazon & sulfoxaflor. Analysis of the data from three trial reveal the influence of "Trial" as a factor on percentage TYLCV was highly significant. In the untreated control, incidence of virus was ranged from a low of 70% at 7 and 14 DAT in the second trial to a high of 100% in each DAT in the third trial. Virus incidence was zero in the flupyradifurone treatment at 3 and 7 DAT for each trial and was not >5% at 14 DAT in any trial (Smith and Giurcanu., 2014).

2.5.A. Bioefficacy of Fluopyram

Succinate dehydrogenase inhibitor fungicides are very much effective in controlling grey mould disease. An In vitro study was carried out to evaluate the efficacy of fluopyram, a novel succinate dehydrogenase inhibitor against all the growth stages of *Botrytis cinerea*, and the protective and curative action of the fungicide against the pathogen was determined on strawberry fruit. Result of the study gives a conclusion that germ tube elongation is the most sensitive stage while

mycelial growth is the least sensitive stage as affected by the fungicide. Excellent protective action of the fungicide against the pathogen achieved at an application rate of 100µg/ml at 96, 48, 36 h before the artificial inoculation of strawberry fruit. At the same time, Effective curative activity of the fluopyram has also been achieved for 24h post inoculation at the same application rate but if the post inoculation is carried out at 48 or 96h, disease control efficacy was modest or low. Study on base line sensitivity proves the fungicide's unimodal nature in all test population (Veloukas and Karaoglanidi, 2011).

2.6 Objective I

1. **Flupyradifurone and Fluopyram persistence study in soil as influenced by moisture regimes of different type of soils and soil amendment**

Soil functioning like a basin **f**or pesticides. A substantial percentage of the foliar applied pesticide are used to reaches in the soil where biological, physical, and chemical factors are interplaying with each other. In order to determine the fate of a pesticides in soil, the above-mentioned strength plays an important role. Environmental sustainability is hugely affected by the fate of this xenobiotics. In order to forecast the residual pesticide concentrations in the soils, information about degradation mechanisms of pesticides and their adsorption nature and circumstances that affect them is essential to assess the potential threat correlated with exposure (https://krishikosh.egranth.ac.in/).

Degradation in Soils

Flupyradifurone field dissipation and laboratory study concludes moderate parent material degradation in soil. Under aerobic conditions, extensive mineralization to CO_2 (up to 52%) and mild levels of non-extractable residues (up to 34%), established by the biological method and tightly bound to the strong (humin) portion of the soil were noted. Two non-volatile metabolites Difluoroacetic acid (DFA) and 6-chloronicotinicacid (6-CNA) were also generated under this condition, which is therefore not feasible to accumulate in the ecosystem under anaerobic or photolytic circumstances (https://docplayer.net).

Rate of Degradation

Degradation in laboratory aerobic soil (in the dark at 20° C)

Parent substance	DT_{50} lab	73 days (geomean US and EU) soils; range 33 – 371 days (all soils)
DFA	DT_{50} lab	61 days (geomean); range 44.9 to 73.6 days
6-CNA	DT_{50} lab	5.3 days (geomean); range 2.9 to 36.6 days

Degradation under field conditions

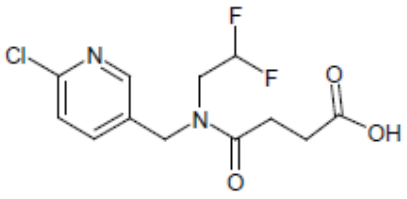
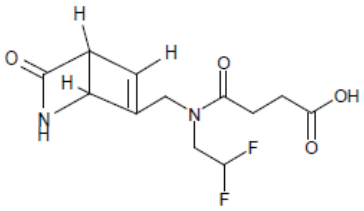
Parent substance	DT_{50} lab	8.3 to 251 days

Fluopyram is hydroxylated in the soil to form the pyridyl-carboxylic acid metabolite, which includes the pyridine ring and the phenyl ring containing benzamide. The metabolization of pyridyl-carboxylic acid to methyl-sulfoxide. Microbial breakdown of the phenyl ring contributes to CO_2 formation. Fluopyram degradation can be characterized as mild to low with a half-life of 162-746 days, under anaerobic conditions fluopyram half-life was greater than 1000 days and therefore fluopyram is regarded steady under oxygen less circumstances in soil. No significant processing products have been identified. (https://vkm.no/)

Degradation in aquatic systems:

In a standardized laboratory condition, fate and behaviour of flupyradifurone in the aquatic system has been studied. A mild amount of degradation of the parent compound and mineralization to CO_2 occurs under aerobic conditions in the biotic system. No significant metabolites were identified; in the overall scheme, DFA (Di Fluro Acetic acid) was created at a maximum of 7 per cent. The calculated DT_{50} in the water phase was reported to be as 9.2 to 53.5 days and in the complete scheme to be 201 to 259 days. The rate of degradation was comparable in an indoor pond research conducted in Germany (DT_{50}-80.6 days in the water). Flupyradifurone was considered to be as stable, under anaerobic laboratory conditions (https://docplayer.net).

Photolysis degradates:

BYI 02960-succinamide	BYI 02960-azabicyclosuccinamide
maximum 40%	maximum 26%
	

Rate of Degradation under laboratory conditions, due to photolysis:

Aqueous buffer photolysis	DT_{50} Lab	13.8 experimental hour
Natural water photolysis	DT_{50} Lab	14 experimental hour

2.7 Objective II

1. **To study the mobility of Flupyradifurone and Fluopyram in soils**

Mobility in soils

Batch equilibrium technique was employed to determine the adsorption constants, K_{oc}, for flupyradifurone and it was concluded that the parent compound has the medium mobility in soil while desorption constants, $K_{d}oc$ indicate potentially stronger adsorption. "Time dependent sorption studies reveal that the sorption of flupyradifurone was increasing increase over time with an ageing factor of 2.6 to 4.4". According to the K_{oc} values, potential high mobility of the major metabolite DFA and medium mobility of 6-CAN has been observed (https://docplayer.net).

Parent Compound	Mean K_{oc}	98.4 mL/g (EU and US soils), 331mL/g (Brazilian soils) + pronounced time dependant sorption
DFA	Mean K_{oc}	6.8 mg/L
6-CAN	Mean K_{oc}	88 mg/L

The adsorption of **fluopyram** can be classified as medium, K_{oc}(ads): 233-400 L/kg. The adsorption of fluopyram-7-hydroxy can be classified as moderate to medium, K_{oc}(ads): 85-149 L/kg. The K_{oc}(des) of the 7-hydroxy metabolite ranged from 237 to 373 L/kg. K_{oc}(des) values were more than two times higher than the K_{oc}(ads) values, indicating a strengthened binding of the compound once adsorbed to the soil (https://vkm.no/).

Exposure to Ground Water

At a concentration of greater than 0.1 µg/L, leaching of fluopyram to the groundwater is not possible (According to, The EU FOCUS). "Swedish scenarios indicate that both fluopyram and the metabolite fluopyram-7-hydroxy have the potential to exceed 0.1 µg/L in groundwater for all crops, except peas" (https://vkm.no/).

Scanty data is available on environmental fate of fluopyram and flupyradifurone dissipation and leaching under subtropical Indian soils. Based on the above literature, a study was undertaken on persistence of fluopyram and flupyradifurone under various moisture regimes and mobility in Indian soils .

Chapter 3

MATERIALS AND METHODS

The objectives of the present investigation were achieved by employing the material and methods which are briefly described below:

3.1 SOIL:

Soil samples from ploughed layer (0-15cm) was collected from the cultivated lands of IARI, New Delhi, PAU, Ludhiana and UBKV, CoochBehar for studying the persistence of flupyradifurone and fluopyram in soil. The soil samples were dried by spreading them on plastic trays and at room temperature the remaining moisture could evaporate. Soil samples were ground with pestle motor, passed through a 2 mm mesh sieve, gathered in a plastic container and stored for various analysis.

Analysis of soil physicochemical properties:

By taking 1:2.5 soil to water proportion, soil pH was measured and blended well with a glass rod and maintained for 30 minutes. Then by using the Control Dynamics pH meter (Model APX 175 E / C) equipped with the installation of calomel glass electrodes (Knauss *et al.,* 1990), pH of the soils has been measured. Walkley and Black process then determined organic carbon content. The Bouyoucos hydrometer technique was used to analyse sand, silt and clay soil fractions (Black, 1965). The physical and chemical characteristics of three soil samples are given below:

Table 2A: Physicochemical soil properties:

Soil properties	IARI Soil	CoochBehar Soil	Ludhiana Soil
Order	Inceptisol	Entisol	Alfisol
Organic carbon (%)	0.312	0.48	0.68
pH	7.73	4.81	8.5
Particle Size Distribution			
Sand (%)	64.9	62.25	60.0
Silt (%)	19.8	19.55	22.2
Clay (%)	15.3	18.20	17.8

3.2 CHEMICALS:

The analytical standards for the experiment was obtained *gratis* from M/s Bayer Crop Science, India with purity percent for Fluopyram 99.4 % and Flupyradifurone 99.4%.

3.2.1 Ordinary solvents:

Hexane with sodium metal has been purified with distillation for 4 hours at 60-80 °C. Acetone was purified by refluxing for 4 hours with potassium permanganate and distilled at 55-60 °C. Single distilled and double distilled water has been used. All solvents used for this study were distilled before using (ethyl acetate, acetonitrile, dichloromethane, acetone , hexane, methanol, water, etc).

3.2.2 HPLC grade solvents:

For HPLC analysis, HPLC grade methanol and HPLC grade acetonitrile were used. From Millipore water purifier (Milli-Q, Model Academic), water was collected for HPLC analysis. All solvents were filtered with 0.45µm filtering paper before HPLC analysis.

3.2.3 Adsorbents:

Silica gel (Qualigens Fine Chemicals, 60-120mesh), charcoal (Merck), sodium chloride, Florisil pesticide grade (Merck), anhydrous magnesium sulfate, PSA (Agellent-part No. 5982-5753), sodium citrate sesquihydrate, sodium citrate dihydrate. Prior to using these adsorbents, silica gel, charcoal and sodium chloride has been washed with acetone and then after filtering, the adsorbents were activated in an oven at 100 °C.

3.2.4 Drying agents:

As a drying agent, anhydrous sodium sulfate was used, washed with acetone and filtrated. Then these washed anhydrous sodium sulfate with acetone was air dried and activated in a Muffle furnace at a temperature near about 450 □ for 4 hr. The adsorbents and drying agents were cooled at room temperature and placed in desiccators.

3.3 INSTRUMENTS:

Spectrophotometer:	Specord 200 (analytic Jena, Germany) UV-Visible spectrophotometer.
Infrared Spectrophotometer	Bruker (Alpha-E) IR spectrophotometer
High performance liquid chromatography (HPLC):	Lachrom HPLC system fitted with Interface D-7000, L-7100 pump and L-7400 UV- Detector and Lachrom auto sampler

Besides these, another equipments like pH meter, horizontal shaker, sonication chamber etc. were also used.

3.4 PREPARATION METHOD FOR STANDARD SOLUTION OF FLUPYRADIFURONE & FLUOPYRAM:

25.25 mg of flupyradifurone (analytical grade 99.4% supplied by Sigma, India) was correctly weighed and was dissolved in acetonitrile which was HPLC grade in a volumetric flask of 25 mL and was made up to the volume to get a stock solution of 1000 µg mL^{-1} (ppm). From this standard solution of 100 µgmL^{-1}, 50 µg mL^{-1}, 20 µg mL^{-1} and 10 µg mL^{-1} were prepared by serial dilution with HPLC grade acetonitrile.

In a similar way, about 25.0 mg of fluopyram (analytical grade 99.7 percent supplied by Fluka Sigma Aldrich India) was precisely weighed and was dissolved in acetonitrile of HPLC grade in a volumetric flask of 25 mL and made up to the volume to get stock solution of 1000 µg mL^{-1} concentration. From this standard solution 100 µg mL^{-1}, 50 µg mL^{-1}, 20 µg mL^{-1} and 10 µg mL^{-1} solutions were prepared by serial dilution with HPLC grade acetonitrile.

These standard stock solutions of two compound were kept in a refrigerator at a temperature of 5 □ for using at the time of experiment. Dilution was done to prepare working solution from these standard solutions whenever needed. For analysis purpose fresh stock solution was prepared after every fifteen days.

3.5 ANALYTICAL METHOD FOR STANDARDIZATION:

3.5.1 Absorbance maxima of Flupyradifurone and Fluopyram determination and HPLC calibration:

Scanning of flupyradifurone and fluopyram in methanol (10 µg mL^{-1}) was carried out on Specord 200 (Analytik Jena, Germany) UV visible spectrophotometer,

wavelength ranging from 400 nm to 200 nm, by using a quartz cuvette with a range of 1 cm, determination λ_{max} of flupyradifurone and fluopyram has been carried out.

For the analytical experiment Merck-Hitachi Lachrom High Performance Liquid Chromatograph with UV detection system and Windows operated integrator was used. Different type of solvent systems like acetonitrile-water (70:30, v/v), 95% acetonitrile-water-0.1%formic acid, acetonitrile-water (80:20, v/v) + 2.5mM ammonium formate were utilized for calibration of the solvent system of the HPLC system.

Different criteria for standardization like linearity, limit of detection, limit of quantification was determined. For determination of the specificity of the method, several criteria like resolution, retention times, limit of detection (LOD), response, limit of quantification (LOQ) was calculated. To check the linearity of the proposed method calibration curve was prepared by plotting areas vs. concentrations of active substances. On the basis of recovery studies of the flupyradifurone and fluopyram accuracy of the method was determined.

To check the sensitivity and linearity of the instruments, standard solutions of different concentrations of 20, 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.001 µg mL^{-1} were prepared. The prepared different concentrations of flupyradifurone and fluopyram were injected into HPLC and a standard curve was prepared by plotting concentration on X-axis and corresponding area response of peak on Y-axis. The following HPLC parameters were standardized for analysis:

Column	Lichrospher, RP-18 (30cm, 4mm, 5µm)
Wavelength of Flupyradifurone (FF, λ_{max})	280 nm
Wavelength of Fluopyram (FP, λ_{max})	280 nm
Run time	10 min
Flow rate	0.5 mL min^{-1}
Injection volume	10 µL
Solvent system	Acetonitrile-water (70:30, v/v) (Isocratic)
Retention time for Flupyradifurone	5.8 min
Retention time for Fluopyram	6.4 min

3.5.2 Fortification of Flupyradifurone and Fluopyram for recovery studies from soil:

10 g of soil was taken in 100 mL beaker (in triplicate) and fortified at 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 20 µg mL^{-1} level by adding 1µl, 5 µl, 10 µl, 50 µl from 10 µg mL^{-1} standard stock solution and 10 µl, 50 µl, 100 µl, 500 µl, 1000 µl and 2000 µl from 100 µg mL^{-1} standard stock solution of flupyradifurone and fluopyram separately. With a glass rod soil was stirred thoroughly and 5 mL of acetone was added to uniformly mix the compounds in soil.

3.5.3 Fortification of Flupyradifurone and Fluopyram for recovery studies from water:

100 mL of water was fortified with flupyradifurone and fluopyram at 0.05, 0.5, 1.0 and 10 µg mL^{-1} level, separately. Liquid-liquid partitioning was done for recovery studies with dichloromethane and ethyl acetate. The samples were partitioned three times with 3×30 mL of each solvent. The combined organic phase was collected and dried through anhydrous sodium sulfate. The extract was evaporated completely under rotary vacuum evaporator and the concentrate was reconstituted in HPLC grade acetonitrile (5 mL).

3.5.4 Extraction procedure for soil:

For the standardization of the extraction procedure, solvent mixtures were used and the following procedure of extraction was evaluated.

3.5.4.1 Method of Dipping and Shaking:

50 mL of different distilled solvents like acetone, acetonitrile, ethyl acetate, dichloromethane and cyclohexane were added to the 10 g of fortified soil samples and shaken on a horizontal shaker continuously for 30 min. The extracted solvents were filtered by utilizing Buchner Funnel which was fitted with a Whatman No. 1 filter paper under vacuum. Shaking and dipping was repeated for two times (2×50 mL of solvent). The solvents were combined, concentrated to 5mL using rotary evaporator and subjected to liquid-liquid partitioning with different polar and non-polar solvents. The concentrate was transferred to separatory funnel and 100 mL of 2% sodium chloride solution was added. Liquid-liquid partitioning was done with dichloromethane for three time (3×30 mL). The DCM extract was concentrated under

vacuum and subjected to column clean up over 5 g neutral alumina sandwiched between layers of 2 g activated anhydrous sodium sulfate. Column was prewashed with 30 mL distilled hexane. The DCM concentrate was loaded on the column and eluted by hexane-acetone (7:3, v/v, 100 mL). The eluant from each column were concentrated under vacuum to dryness and reconstituted with 5 mL of acetonitrile for HPLC analysis.

3.5.4.2 Single step column extraction:

In a beaker 10 g of fortified soil sample was taken and 7-10 drops of ammonia was added. Soil was properly stirred with glass rod and kept undisturbed for 2-3 hrs. On observing no smell of ammonia activated charcoal (0.5 g) and florisil (0.5 g) were mixed with soil. A clean glass column (30 cm × 2 cm) was taken and plugged at bottom with cotton. 2 g of anhydrous sodium sulfate was added into the column followed by soil mixture and another layer of 2 g anhydrous sodium sulfate was added to the column. After filling the column with appropriate materials cotton was positioned at the top of all the layers of the column. The column was pre washed with hexane, followed by elution with hexane-acetone solvent system (100 mL, 7:3 v/v). The eluted solvent was collected, concentrated and reconstituted in 5mL of HPLC grade acetonitrile and kept aside for HPLC analysis.

3.6 CLEAN UP METHOD FOR STANDARDIZATION:

3.6.1 Liquid-liquid partitioning with DCM (dichloromethane):

From the section 3.5.4.1 extract was evaporated on a rotary vacuum evaporator and moved to a separating funnel of 250 mL. It was accompanied with 100 mL of saline solution (2% NaCl) and 30 mL of dichloromethane. With pressure discharge, the separatory funnel was carefully shaken for one minute and then permitted to stand (10 minutes) until two layers were separated. A layer of anhydrous sodium sulphate was carried through the bottom layer and retained in a reagent bottle. With 30 mL of dichloromethane (30×2 mL), the aqueous layer remaining in the separating funnel was again partitioned for two more occasions. After passing through anhydrous sodium sulfate, the organic layers were again assembled in the same conical flask. Again, anhydrous sodium sulfate was washed and retrieved in the same container with 10mL of dichloromethane. It was placed in the refrigerator after full evaporation of the organic layer until the column was cleaned up.

3.6.2 Liquid-liquid partitioning with ethyl acetate:

After extraction, a rotary vacuum evaporator evaporated the organic extract and moved it to a separating funnel of 250 mL. It was furnished with 100 mL of saline solution (2% NaCl) and 30 mL of ethyl acetate. With pressure discharge, the separatory funnel was carefully shaken for one minute and then permitted to stand (10 minutes) until two layers were separated. A layer of anhydrous sodium sulphate passed through the lower layer and was retained in a reagent flask. The aqueous layer remaining in the separatory funnel was again partitioned with 30 mL of ethyl acetate (30×2 mL) for two more occasions, passed through anhydrous sodium sulfate and held away for cleaning.

3.6.3 Modified QuEChERS method for soil:

10 g of fortified sample was taken in a centrifuge tube, 10 mL of acetonitrile was added to the soil and vortexed for 3 min. 6 g magnesium sulphate ($MgSO_4$), 1.5 g sodium chloride (NaCl), 0.750 g disodium citrate sesquihydrate (Na_2HCit, $1.5H_2O$) and 1.5 g sodium citrate dihydrate (Na_3Cit, $2H_2O$) were added and shaken by a vortex to prevent salt agglomeration. Next, the mixture was sonicated in an ultrasonic bath for 1 min and samples were then centrifuged at 4500 rpm for 10 min. 1.5 mL of the supernatant was removed and transferred to the clean-up tube containing 50 mg of PSA, 150 mg of $MgSO_4$ and 50 mg of C-18. The tubes were capped tightly and shaken on the vortex for 1 min followed by centrifugation at 4500 rpm for 5 min. Finally, 1mL supernatant was collected and filtered through micro filter and analysed with the help of HPLC. (Correia□Sá *et al*., 2012)

3.7 Confirmation of Flupyradifurone and Fluopyram:

3.7.1 Thin layer chromatography (TLC):

Thin layer chromatography was done by using an aluminium plate coated with silica gel (20 cm x 5 cm), which was pre-activated at 105 ^oC in an oven. To find out different polarities of flupyradifurone and fluopyram, solvent mixtures, ethyl acetate-methanol (9:1 ratio, v/v) was used for evolving TLC plates. The small amount of analytical grade standard compound was diluted in acetone and using capillary tubes, it was spotted on a TLC plate. Iodine vapour chamber was used as a visualizing agent.

$$\text{Retention factor } (R_f) = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

3.7.2 IR spectroscopy

On the IR spectrophotometer Bruker (Alpha-E), the IR spectra was recorded in the range 4000-400 cm^{-1}. KBr was used to investigate samples of the IR spectrophotometer.

3.8 PERSISTENCE OF FLUPYRADIFURONE & FLUOPYRAM IN SOIL:

3.8.1 Field capacity moisture level determination:

For determining field capacity, a soil column was produced in a 100 mL measuring cylinder. With such a 100 mL measuring cylinder, 138 g of soil was tightly packed, 10 ml of water was poured into the column and maintained without disturbing it. Water penetrated the soil column and moisturized the soil continuously. After 60 minutes the water level percolated down the soil column was recorded and the necessary water level to wet the soil was provided.

3.8.2 Soil treatment

Three different soil types used in the experiment were fortified separately with flupyradifurone and fluopyram at 10 µg mL^{-1} level. Soils were fortified at 10 µg g^{-1} (ppm) level, by using 3 mL of 100 µg mL^{-1} standard solution of flupyradifurone and fluopyram in 300 gm soil in a beaker. Compounds were correctly blended into soils by the aid of a glass rod and kept for 24 hours uninterrupted. The uniformity of the treated soils was verified. In terms of residues, samples were triplicated, and then every soil treated was assumed to be homogeneous.

3.8.3 Persistence study

The persistence of flupyradifurone and fluopyram was studied at 10 µg g^{-1} (ppm) fortification level in three different soils collected from Inceptisol (IARI soil), Alfisol (Ludhiana soil), and Entisol (CoochBehar soil) to evaluate the effect of moisture and soil type on dissipation of flupyradifurone and fluopyram. Under three distinct moisture regimes viz submerged, field capacity and air-dry condition, the effect of moisture from three distinct locations soil was explored. Similar experiments were conducted at ambient temperature (25-28 °C) in FYM (from the IARI farm)

under FC (field-capacity) moisture regime. The sampling was performed for all three circumstances in three distinct soils and FYM at 0, 1, 3, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120 days.

3.8.4 Effect of moisture regime in soils and manures:

Under air-dry, field capacity and submerged condition, effect of different moisture regimes on dissipation of flupyradifurone and fluopyram was studied at 10 µg g^{-1} (ppm) fortification level of three different types of soils and FYM. After weighing, 10 grams of control soil and 10 g of treated soil samples were transferred to a 25 ml beaker. A calculated amount of water has been added into the soil and manure before studying the impact of distinct moisture regimes. To maintain the field capacity moisture level, 17-18% water for Inceptisol (IARI soil) and 25% for Entisol (CoochBehar soil) were added while in case of submerged soil condition enough water was added to raise the level of water to about 3cm above the soil surface. In air dry condition no water was added.

3.10 LEACHING STUDIES:

Leaching experiments were conducted in packed soil columns of Inceptisol (IARI soil), Alfisol (Ludhiana soil) and Entisol (CoochBehar soil) with flupyradifurone and fluopyram.

3.10.1 Packed soil column:

The leaching of flupyradifurone and fluopyram in three different soils, Inceptisol (IARI soil), Alfisol (Ludhiana soil) and Entisol (CoochBehar soil) were studied in polythene columns in laboratory. Polythene columns (50 cm × 2.1 cm), were packed with air dried and sieved soil up to 25 cm length (IARI soil weight 220 g, Ludhiana soil 227g and CoochBehar soil 223 g). With 10 µg g^{-1} flupyradifurone and fluopyram, 2 g of soil was fortified individually and blended with a glass rod and retained untouched. The leaching activities of flupyradifurone and fluopyram were done in triplicate under continuous and discontinuous flow conditions. Using a pin, tiny holes were created to retrieve the leachate at the lower end of the polythene columns. The lower end of the column was dipped into the water overnight to lift the water well into the column of the soil by capillary action. The next day, the column was suspended vertically to drain surplus water. At the top of the soil columns, earlier fortified soil (2 g) was systematically distributed and leaching began. Water was

permitted to flow under natural circumstances. The leaching experiment was carried out in continuous flow, where 400ml of water simulating 1156 mm rainwater passed through the column. In another discontinuous state of condition, columns were subjected to different amount of water i.e. 40, 80, 160 and 240 mL was added simulating 51.92, 103.85, 207.71 and 415.42-mm rainfall to observe the leaching behaviour of flupyradifurone and fluopyram in Inceptisol (IARI soil), Alfisol (Ludhiana soil) and Entisol (CoochBehar soil)

Column leachate from each continuous and discontinuous column was retrieved, filtered and diluted with 100 mL of saturated sodium chloride solution and extracted with 30 mL of dichloromethane (330 mL) by liquid-liquid partitioning. The lower layers of dichloromethane were collected, dried over anhydrous sodium sulfate and concentrated. The soil column was sliced horizontally into five 5 cm cores each (0-5, 5-10, 10-15, 15-20, 20-25 cm) after complete water addition. Every core was airdried and weighed. Extraction was carried out using acetone by dipping and shaking technique. Samples were processed as per procedure given in section 3.5.4.1 (standardized method) and estimated for the flupyradifurone and fluopyram residue using HPLC.

3.11 Calculation and data analysis:

After analysis with HPLC, the concentration of residues of flupyradifurone and fluopyram and percent recovery of these two compounds were calculated using this following formula:

$$\textbf{Concentration (µg g}^{-1}\textbf{)} = \frac{\text{Sample peak area} \times \text{standard concentration (µg mL}^{-1}) \times \text{extract volume (mL)} \times \text{standard injected volume (mL)}}{\text{Standard peak area} \times \text{weight of sample processed (g)}}$$

$$\textbf{Recover percentage} = \frac{\text{Recovery amount (µg g}^{-1})}{\text{Fortification level (µg g}^{-1})} \times 100$$

Theoretically, there has been a trend of logarithmic decrease of flupyradifurone and fluopyram residues. The concentration of residues dissipated per unit time is proportional to the total concentration of residues present at a specific time, provided that the entire lot is exposed uniformly to the different agencies of

dissipation such as degradation reactions, weathering and metabolism etc. (Hoskin, 1961).

The residue data of flupyradifurone and fluopyram obtained from persistence experiment under laboratory conditions was subjected to first order rate kinetics:

$$\delta C/\delta t = KC$$

Where, C is concentration and t is time. This equation is a straight-line equation:

$$\text{Log } C_t = \text{Log } C_0 - K_t/2.303$$

Where, C_t is the concentration after a time lapse't'; C_0 is apparent initial concentration; 't' is time lapsed, K is dissipation constant. The Log (residues) data and time was subjected to linear regression analysis (Y = a - bX) and calculation of K value was done by the following formula:

$$K = (b \times 2.303)$$

In some cases, it has also been observed that residues of pesticide dissipated in a biphasic manner. Representation of this two-phase process can be done by nonlinear model (Jin and Webster, 1998). The equation is,

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

where, C_1= apparent initial deposits obtained for fast dissipation phase

C_2= apparent initial deposits obtained by extrapolating the line to zero time for slow dissipation phase.

K_1 and K_2 = dissipation rate constants for fast and slow phases.

3.11.1 Residual half-life calculation (RL_{50} or $t_{1/2}$):

The half-life of residue can be defined as the time (in days or hours) required to reduce the concentration of pesticide residues to half of its original concentration deposited. Hoskins (1961) proposed the following equation for calculation of half-life.

$$RL_{50} \text{ or } t_{1/2} = \frac{\text{Log } 2}{b} \quad \text{or} \quad RL_{50} \text{ or } t_{1/2} = \frac{0.693}{k}$$

Chapter 4

RESULTS

The findings of the current flupyradifurone and fluopyram research are provided under the following headings and debated:

4.1 FLUPYRADIFURONE & FLUOPYRAM CONFORMATIONS:

Structure conformation and purity of Flupyradifurone and fluopyram were performed by TLC and IR observation. The melting point was 68 °C and 118 °C compared to the literature value of 69 □ and 118 □, respectively for flupyradifurone and fluopyram (Jeschke et al., 2015).

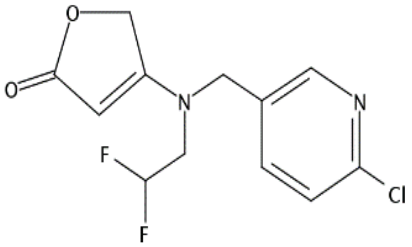


Figure 6C: Structure of Flupyradifurone

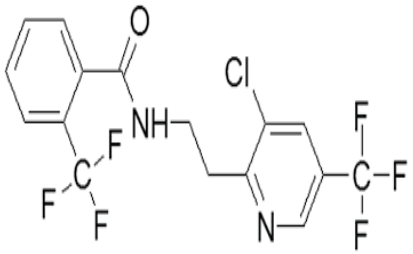


Figure 6D: Structure of Fluopyram

4.1.1 THIN LAYER CHROMATOGRAPHY (TLC):

Thin Layer Chromatography of flupyradifurone and fluopyram was conducted using the solvent system of methanol: ethyl acetate (1:9), 0.52 and 0.60 values were reported.

4.1.2 Infra- Red spectrum (using KBr pellet method) of fluopyram (Figure 7C):

3273 cm^{-1} (-NH-sym), 3084.58 cm^{-1} (-NH-assym), 1642.18 cm^{-1} (Carbonyl-CO), 1131.5 cm^{-1} (Cyano-CN), 1056.83 cm^{-1} (-C-F).

4.1.3 Infra- Red spectrum (using KBr pellet method) of flupyradifurone (Figure 7D):

1784.12 cm^{-1} (-CO-sym, five membered ring), 99.84 cm^{-1} (-C-F-sym), 1460.07 cm^{-1} (-CH-sym), 1715.30 cm^{-1} (-CO-stretching), 778 cm^{-1} (-C-Cl).

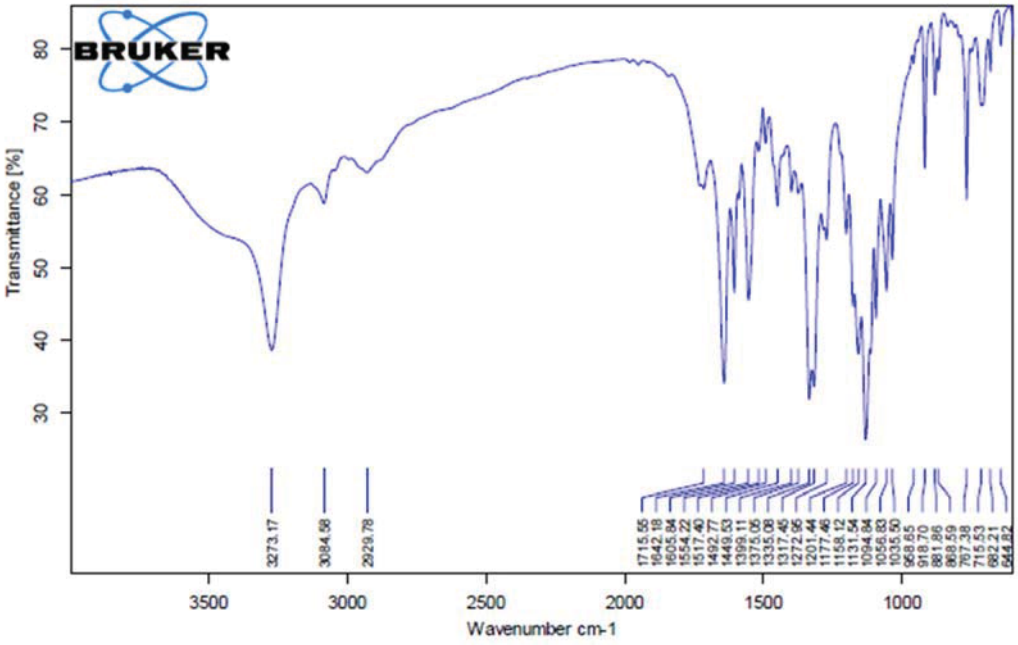


Figure 7C: IR spectra of Fluopyram

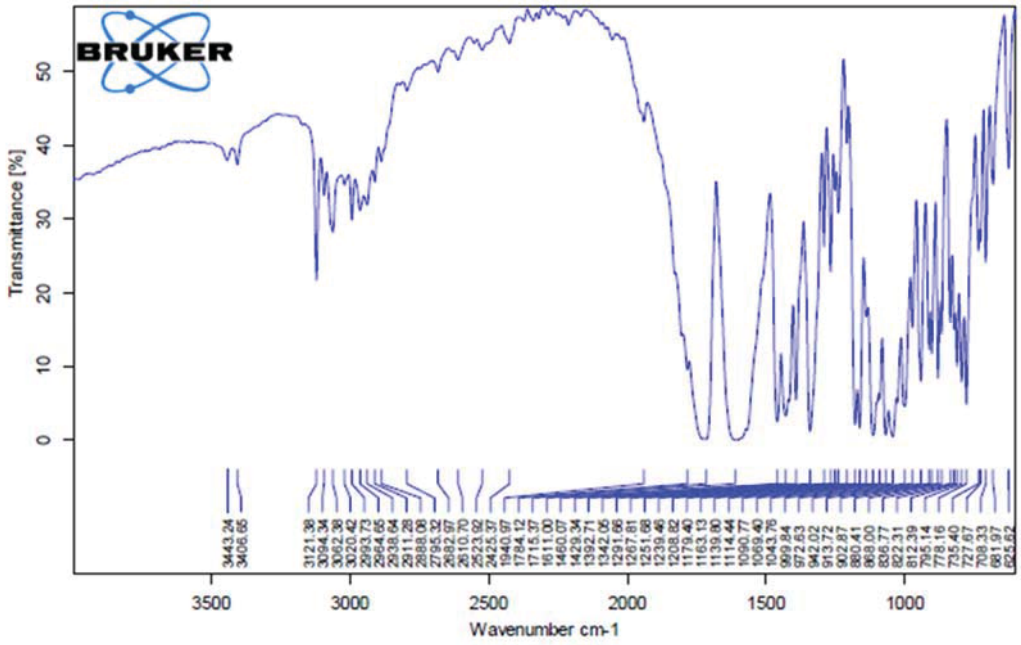


Figure 7D: IR spectra of Flupyradifurone

4.2 STANDARIZATION OF METHODOLOGY:

Two significant criteria for standardization of flupyradifurone and fluopyram analytical methodology and evaluation are-

i) Standardization of analytical method.

ii) Determining the effectiveness of extraction and clean-up procedure

4.2.1 Standardization of analytical technique:

Absorbance maxima (λ_{max}) of flupyradifurone and fluopyram:

In Specord 200 (Analytical Jena, Germany), the standard solution of flupyradifurone and fluopyram (10 ppm) was analyzed from 400 nm to 200 nm range in methanol (HPLC grade). At 280 nm wavelength, the absorption of flupyradifurone and fluopyram was the highest individually. As the flupyradifurone and fluopyram maximum absorbance was 280 nm, the HPLC-UV assessment was performed at the specified wavelength.

4.2.2 HPLC conditions standardization for flupyradifurone and fluopyram:

Flupyradifurone and fluopyram, are to be estimated by "Merk Hitachi LachromL-7400 High Performance Liquid Chromatograph system equipped with UV detector and Windows operated integrator which was used for analysis". UV detector was used to optimize and standardize HPLC assessment circumstances of flupyradifurone and fluopyram.

a) Suitable column selection for HPLC:

Flupyradifurone and fluopyram have been analyzed on the "stainless steel column (5µm, 4 mm, and 30 cm) of Lichrospher Reverse Phase C-18".

b) Mobile phase for HPLC:

To optimized the flupyradifurone and fluopyram evaluation technique different mobile phases have been used. Mixed solvent systems used in the study- "i) 95% acetonitrile-water-0.1% formic acid + 2.5 Mm ammonium formate, ii) milli-Q water, iii) water-acetonitrile (30:70 v/v)". As water-acetonitrile (30:70 v/v) mobile phase provided better resolution and appropriate retention time in case of flupyradifurone and fluopyram, it was chosen to identify two compounds.

c) UV detector's Wavelength determination in HPLC:

The maximum absorbance (λ_{max}) of 280 nm for Flupyradifurone and Fluopyram were used by using UV detector in the HPLC system.

d) Mobile phase flow rate:

Flow rate of 0.5 mL/ min for the mobile phase was considered suitable for the assessment of the compounds, flupyradifurone and fluopyram and retained during the research.

e) Flupyradifurone and Fluopyram Quantification:

Distinct concentration of flupyradifurone and fluopyram has been injected into the High Performance Liquid Chromatography system to quantify both the compounds using the area of peak provided in the table (3A). The linearity of flupyradifurone's HPLC response is provided in Table (3A) along with a calibration curve plotted flupyradifurone concentration on the X-axis and peak area on Y-axis.

Table (3A). Linearity of response of HPLC of Flupyradifurone:

Level of Fortification (ppm)	Avg. area of peak
20	20622991
10	1017807
5	512000
2.5	238901
1	98197
0.5	50125
0.1	19701
0.05	15900
0.01	1950
0.005	960

Although the flupyradifurone linearity range was 20-0.005 ppm, the concentration of flupyradifurone at 0.005 ppm level, provided a well-resolved and definite peak in the High Performance Liquid Chromatography instrument. The quantification threshold (LOQ) was 0.05 ppm and the detection limit (LOD) was 0.005 ppm based on retrieval research. Similarly, the results were quantified in terms of area of the peak by injecting distinct fluopyram concentrations is presented in Table (3B).

Table (3B). Linearity of response of HPLC of Fluopyram:

Level of Fortification (ppm)	Avg. area of peak
20	2020851
10	1009026
5	504004
2.5	251500
1	111057
0.5	49575
0.1	11109
0.05	5789
0.01	1940
0.005	940

4.2.3 Study of recovery from water samples by using various organic solvents:

For the retrieval research of flupyradifurone and fluopyram, two distinct types of organic solvents such as ethyl acetate and dichloromethane (DCM) were applied in the LLP (liquid-liquid partitioning) of both flupyradifurone and fluopyram from samples of water. Four distinct types of fortified standard solution such as 10, 1, 0.5 and 0.05 ppm were used in the recovery research of entire experiment.

The retrieval proportion of flupyradifurone in the initial extraction with dichloromethane ranged from 96-75 %, with 95.8 %, 86.8 %, 80.4 %, and 75.2 % at 10, 1, 0.5 and 0.05 ppm level, correspondingly. Similarly, the proportion of flupyradifurone regeneration using ethyl acetate ranged from 89% to 72%, with 89.1%, 80.5%, 72.4%, and 72.5% at 10, 1, 0.5 and 0.05 ppm, respectively. It was evident from this result that dichloromethane provided a higher proportion of regeneration of flupyradifurone compared to ethyl acetate.

Also, the extraction of compounds from water samples by LLP for fluopyram, ethyl acetate and dichloromethane was used. In the foremost extraction with dichloromethane, the retrieval fraction of fluopyram ranged between 96-72%, with 96.1%, 88.5%, 80.4% and 72.3% at 10, 1, 0.5 and 0.05 ppm level, individually. Similarly, retrieval fraction of fluopyram by means of ethyl acetate ranged between

85-71%, with 85.4%, 80.8%, 75.3% and 71.4% at 10, 1, 0.5 and 0.05 ppm level, correspondingly. It became apparent from this consequence that DCM (dichloromethane) gave more retrieval of fluopyram as compared to EtOAc (ethyl acetate).

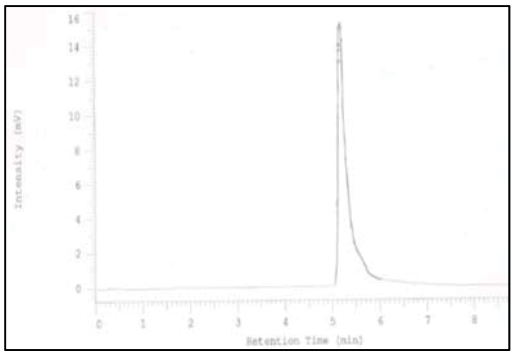


Figure 9A: HPLC chromatogram for Flupyradifurone at 10 ppm level

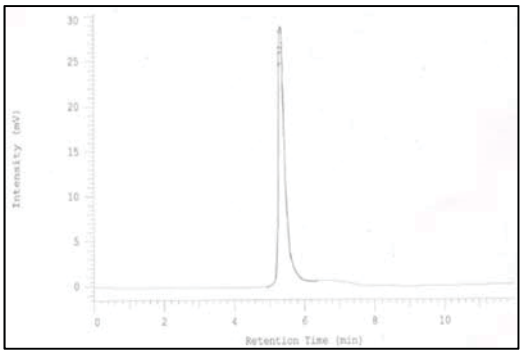


Figure 9B: HPLC chromatogram for Fluopyram at 10 ppm level

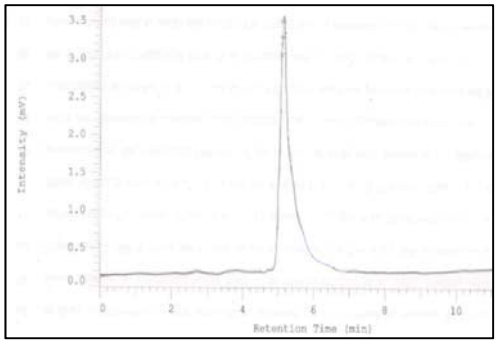


Figure 9C: HPLC chromatogram for Flupyradifurone at 1 ppm level

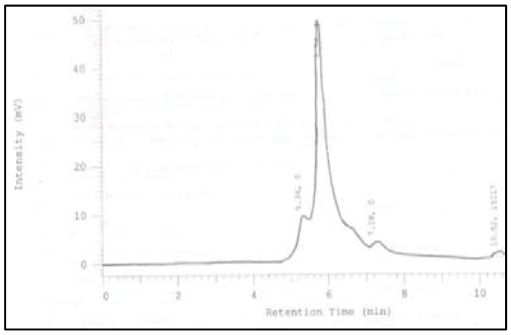


Figure 9D: HPLC chromatogram for Fluopyram at 1 ppm level

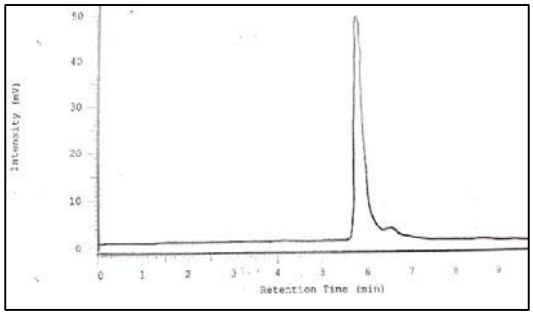


Figure 9E: HPLC chromatogram for Flupyradifurone at 0.05 µg mL^{-1} level

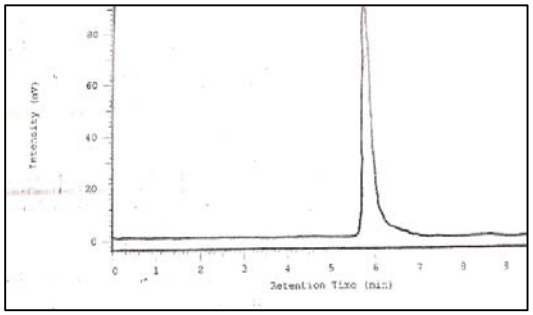


Figure 9F: HPLC chromatogram for Fluopyram at 0.05 µg mL^{-1} level

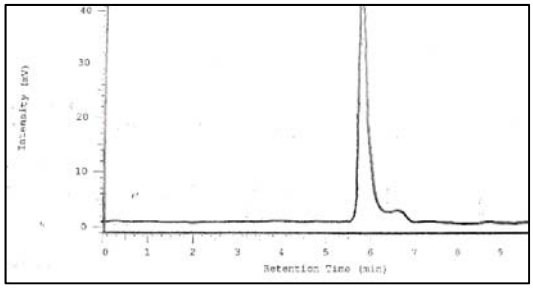


Figure 9G: HPLC chromatogram for Flupyradifurone at 0.1 ppm level

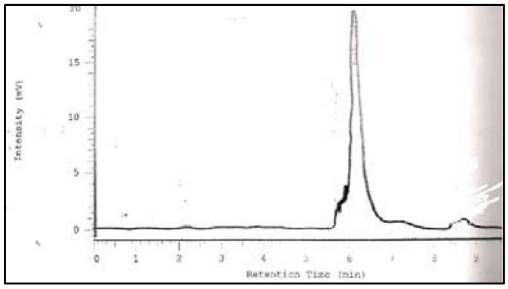


Figure 9H: HPLC chromatogram for Fluopyram at 0.1 ppm level

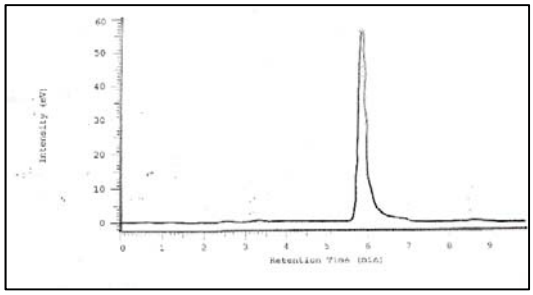


Figure 9I: HPLC chromatogram for Flupyradifurone at 0.01 ppm level

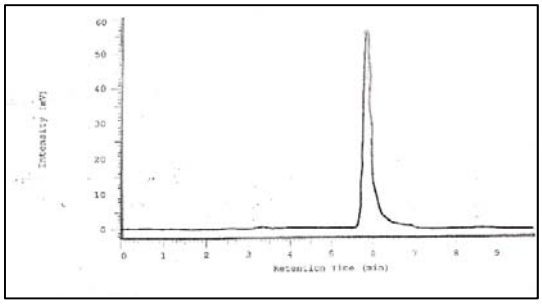


Figure 9J: HPLC chromatogram for Fluopyram at 0.01 ppm level

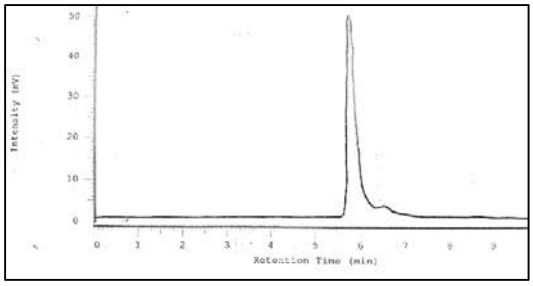


Figure 9K: HPLC chromatogram for Flupyradifurone at 0.005 µg mL^{-1} level

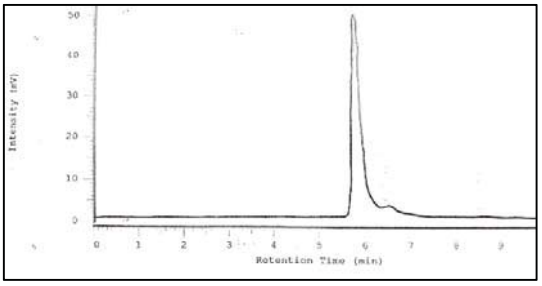


Figure 9L: HPLC chromatogram for Fluopyram at 0.005 µg mL^{-1} level

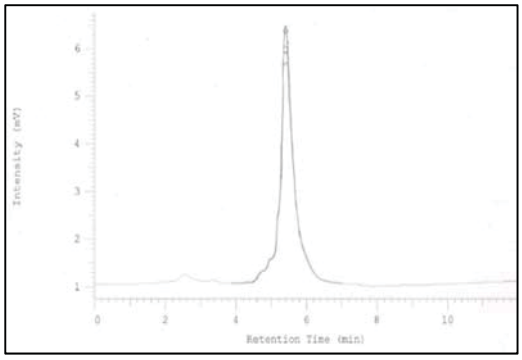


Figure 9M: HPLC chromatogram for Flupyradifurone fortified soil (0.005 µg g^{-1})

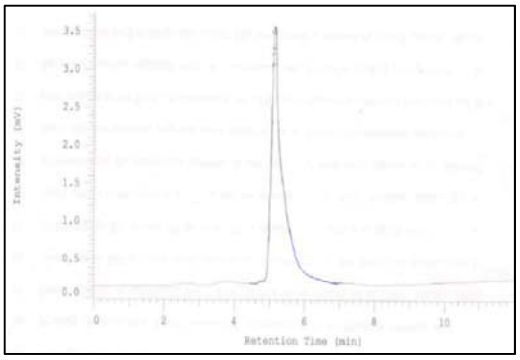


Figure 9N: HPLC chromatogram for Fluopyram fortified soil (0.005 µg/g)

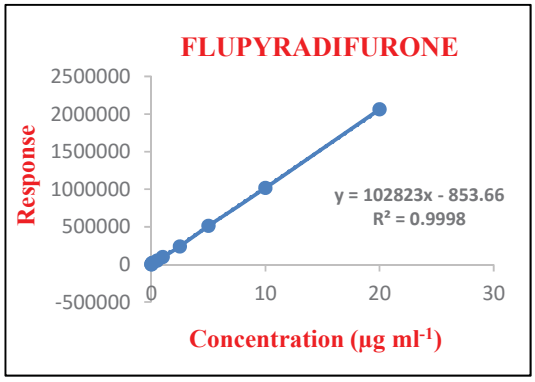


Figure 9O: Detector response vs Conc. curve for Flupyradifurone

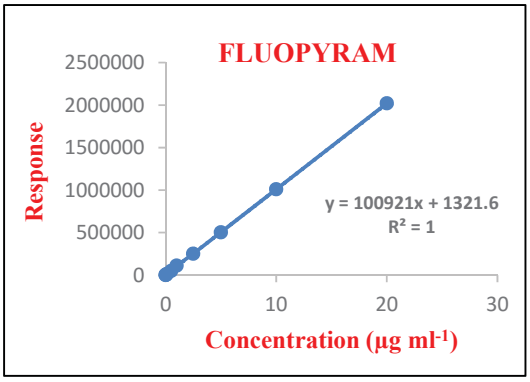


Figure 9P: Detector response vs conc. curve for Fluopyram

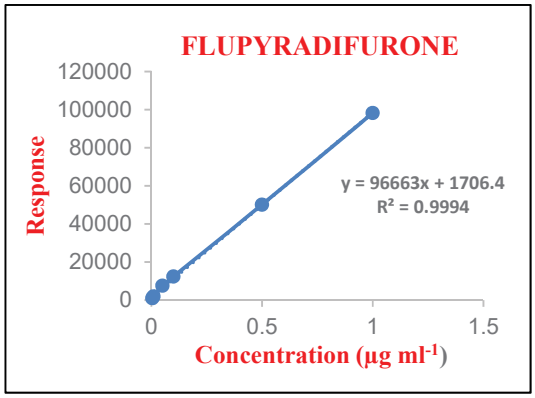


Figure 9Q: Detector response vs Conc. curve for Flupyradifurone

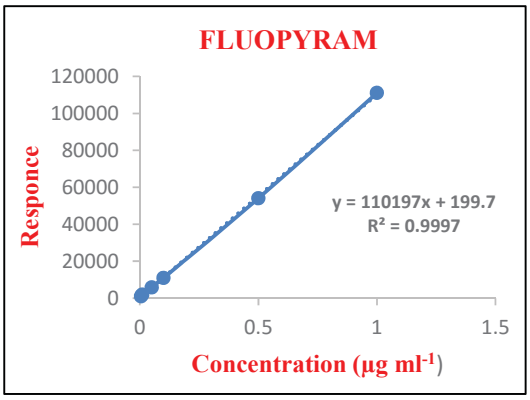


Figure 9R: Detector response vs conc. curve for Fluopyram

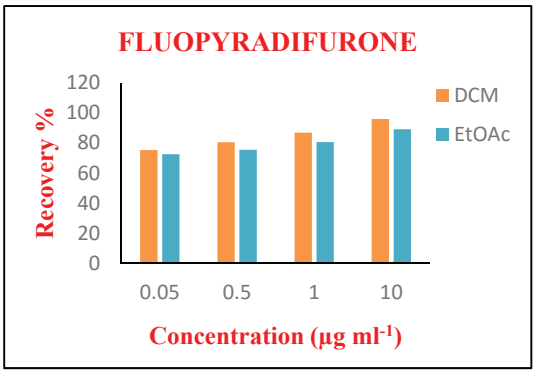


Figure 9S : recovery % of Flupyradifurone using different solvents

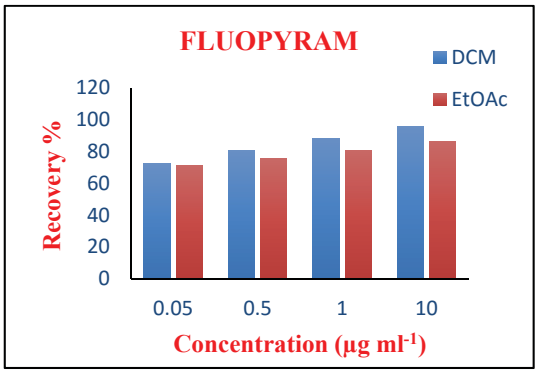


Figure 9T : recovery % of Fluopyram from water using different solvents

4.2.3 Studies in soil recovery using various organic solvents:

To record the suitable extraction solvent and boost extraction effectiveness, five distinct solvents, such as, dichloromethane, acetonitrile, cyclohexane, ethyl acetate and acetone were used for the acquisition of flupyradifurone and fluopyram from 1 ppm spiked soil samples. Recovery % for both the compounds were 98.5, 95.6, 94.5, 92.2, 92% and 97.5, 94.5, 92.4, 92.1, 89.8% for acetone, acetonitrile, ethyl acetate, dichloromethane and cyclohexane, individually.

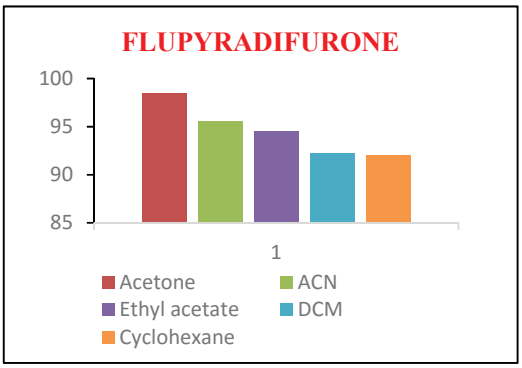


Figure 9U: recovery % of Flupyradifurone soil using different solvents

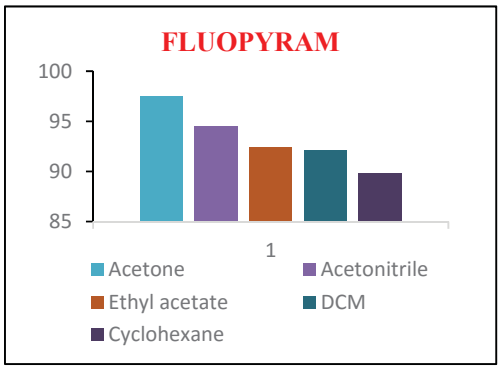


Figure 9V: recovery % of Fluopyram from soil using different solvents

4.2.4 Analytical methodology standardization for samples of soils:

Three distinct kinds of extraction techniques were used in order to extract the two pesticides namely, flupyradifurone and fluopyram from the soil. The procedures of extraction are i) The most prevalent technique of dipping, shaking and clean up

using silica gel, ii) Modified QuEChERS method and iii) single step extraction method at 0.05, 0.5. 1.0 and 10.00 ppm level of fortification (Figures 9S & 9T).

In traditional "dipping and shaking" technique for flupyradifurone and fluopyram, the per cent mean retrievals were 84.5 and 81.5% at 0.05 ppm spiking level, 86.9 and 87.4% at 0.5 ppm spiking level, 92.5 and 90.6% at 1 ppm spiking level and 96 and 92.4% at 10 ppm spiking level, individually.

Obtained mean recovery of both the compounds by using single step extraction technique were 80.6 and 76.4% at 0.05 ppm level of fortification, 81.5 and 80.2% at 0.5 ppm level of fortification, 85.8 and 84.4% at 1 ppm level of fortification, 90 and 86.5% at 10 ppm level of fortification, correspondingly.

In the extraction and washing of samples of soils using modified QuEChERS technique for flupyradifurone and fluopyram, the per cent mean retrievals obtained were 80.5 and 81.1% at 0.05 ppm level of fortification, 85.2 and 84.5% at 0.5 ppm level of fortification, 90.9 and 88.3% at 1 ppm level of fortification, 94.8 and 90% at 10 ppm level of fortification, individually. The dipping and shaking technique furnished the largest retrieval proportion of flupyradifurone and fluopyram at 10 ppm among these three extraction processes. This technique has therefore been used for residue assessment from the soil matrix in all experimental study for flupyradifurone and fluopyram. The recovery information obtained using distinct soil sampling techniques expressed that "dipping and shaking" using acetone as a solvent and thereafter, column cleaning using silica gel resulted in a greater recovery than the single step technique.

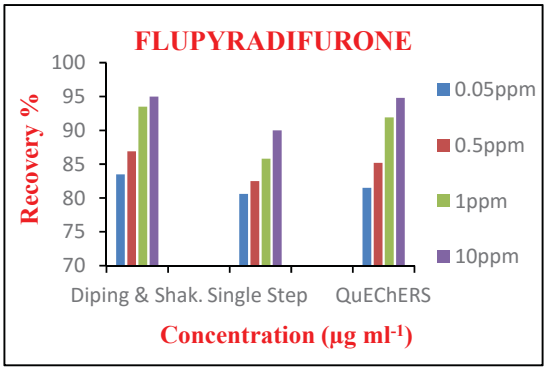


Figure 9AA: recovery % using different extraction methods of Flupyradifurone

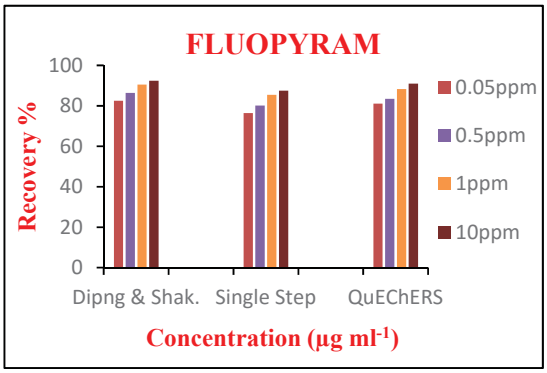


Figure 9AB: recovery % using extraction methods of Fluopyram

4.3 FLUPYRADIFURONE & FLUOPYRAM PERSISTENCE IN SOILS:

The analysis of flupyradifurone and fluopyram dissipation pattern in various moisture circumstances was noted at 10 ppm fortification level under submerged, field ability and dry condition. Flupyradifurone and fluopyram residues were evaluated under separate moisture regimes from fortified soil samples.

4.3.1 Soil persistence study of Flupyradifurone and Fluopyram at air-dry condition:

Residue information of both the compounds has been shown in Table (1, 2, 3, 4, 5 & 6) (Appendix). Up to 120 days residues of both the compounds were identified under air dry moisture condition.

In air-dry moisture condition of Inceptisol degradation of both the compounds were taken place at a slow rate. Fortification level for samples of soils was 10 ppm. At 60, 45, 30, 10 and 5 days fortification, residues of both the compound were detected to be as 7.76, 8.22, 8.75, 9.35, 9.51 ppm and 6.45, 7.10, 7.43, 8.88 and 9.30 ppm respectively. Flupyradifurone and fluopyram degradation pattern followed 1st order rate kinetics. Half-life of both the compounds were reported to be as 200 and 107.5 days in Inceptisol. Regression equation corresponding to 1st order rate kinetics has been depicted in Table 4A.

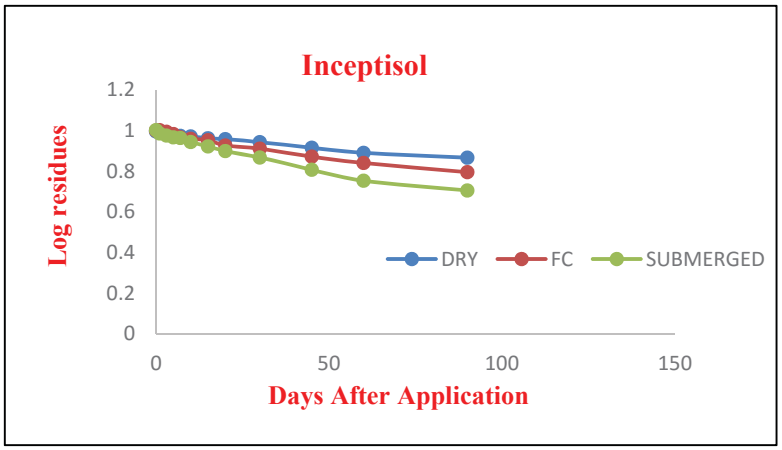


Figure 10A: Persistence of Flupyradifurone in Inceptisol under different moisture regimes.

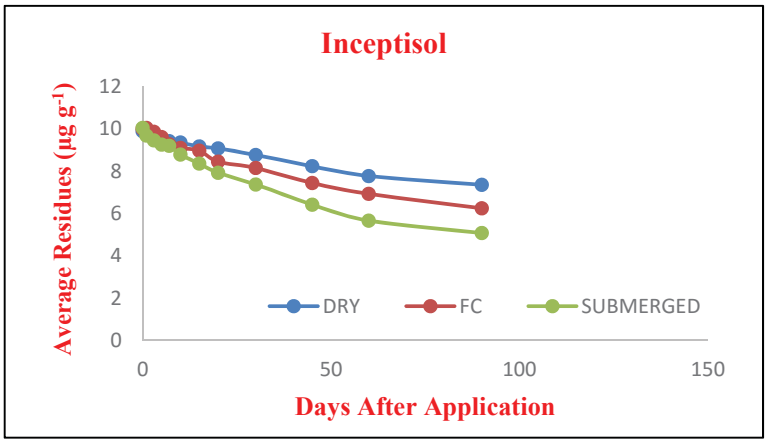


Figure 10B: Persistence of Flupyradifurone in Inceptisol under different moisture regimes

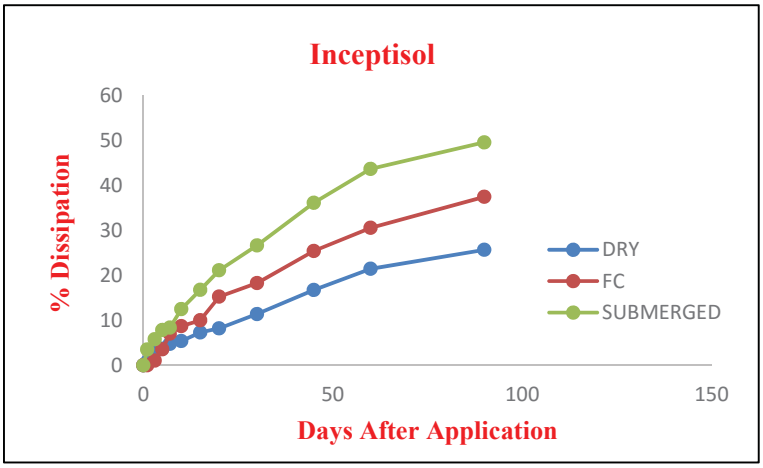


Figure 10C: % dissipation of Flupyradifurone in Inceptisol under different moisture regimes

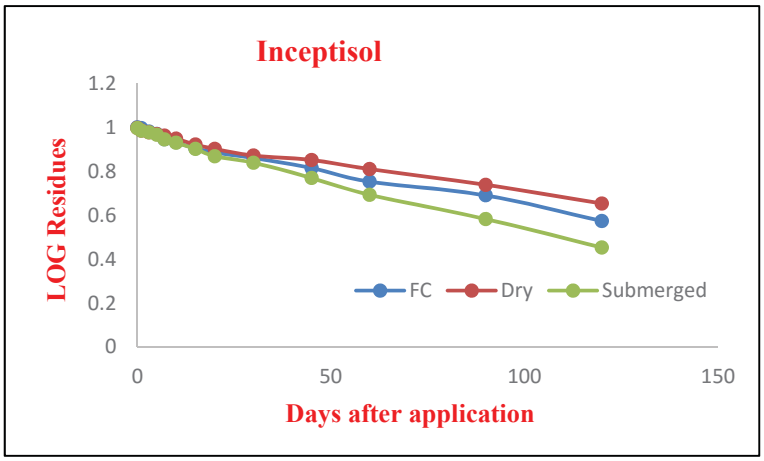


Figure 10D: Persistence of Fluopyram in Inceptisol under different moisture regimes

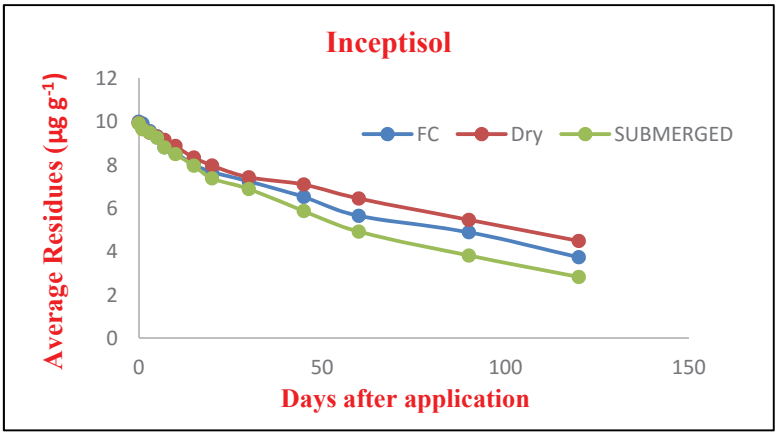


Figure 10E: Persistence of Fluopyram in Inceptisol under different moisture regimes

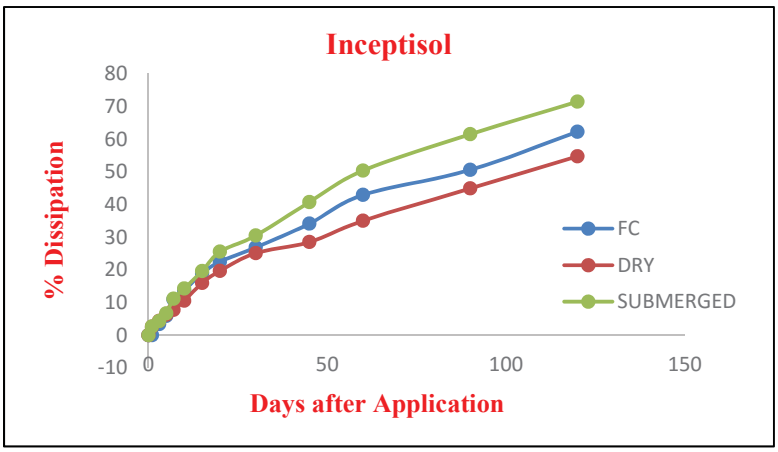


Figure 10F: % dissipation of Fluopyram in Inceptisol under different moisture regimes

Table (4A): Regression equation for first order dissipation of Flupyradifurone and Fluopyram in Air Dry condition of Inceptisol :

Chemicals used	Spiking level (ppm)	Regression eq.	R^2	K	Half-life (days)
Flupyradifurone	10	y = -0.0015x + 0.9867	0.98	0.003465	200.00
Fluopyram	10	y = -0.0053x + 0.9895	0.98	0.006447	107.5

Likewise, in Entisol, dissipation rate of flupyradifurone and fluopyram is also slow in air-dry moisture condition. Fortification level for samples of soils was 10

ppm. At 60, 45, 30, 10 and 5 days fortification, residues of both the compound were detected to be as 8.36, 8.73, 9.05, 9.53, 9.67 ppm and 6.40, 7.22, 7.69, 8.70, 9.35 ppm respectively. Flupyradifurone and fluopyram degradation pattern followed 1st order rate kinetics. Half-life of both the compounds were reported to be as 273.63 and 115.75 days in Entisol. Regression equation corresponding to 1st order rate kinetics has been depicted in Table 4B.

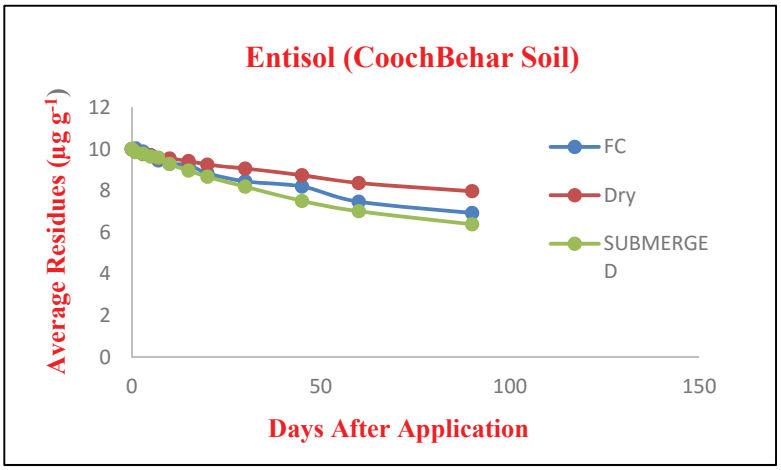


Figure 11A: Persistence of Flupyradifurone in Entisol under different moisture regimes

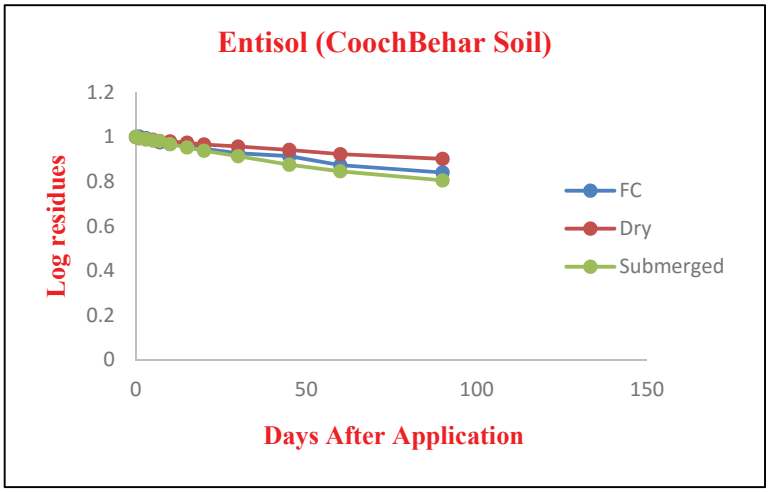


Figure 11B: Persistence of Flupyradifurone in Entisol under different moisture regimes

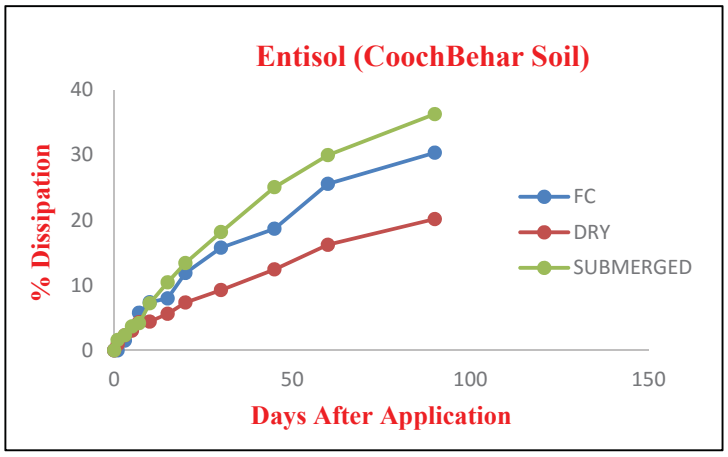


Figure 11C: % dissipation of Flupyradifurone in Entisol under different moisture regimes

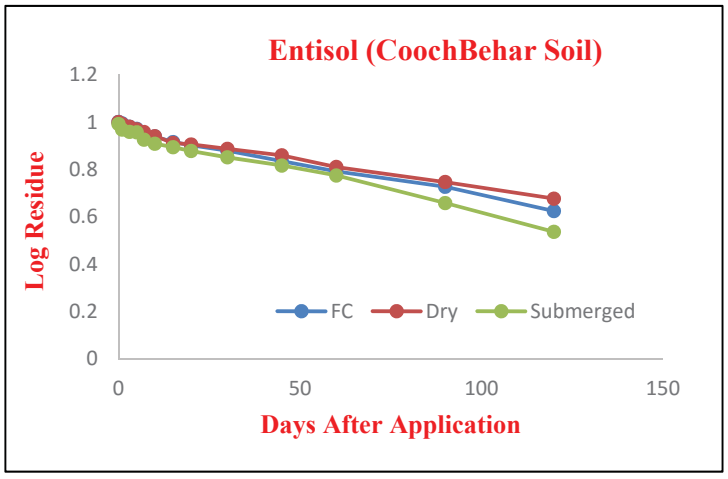


Figure 11D: Persistence of Fluopyram in Entisol under different moisture regimes.

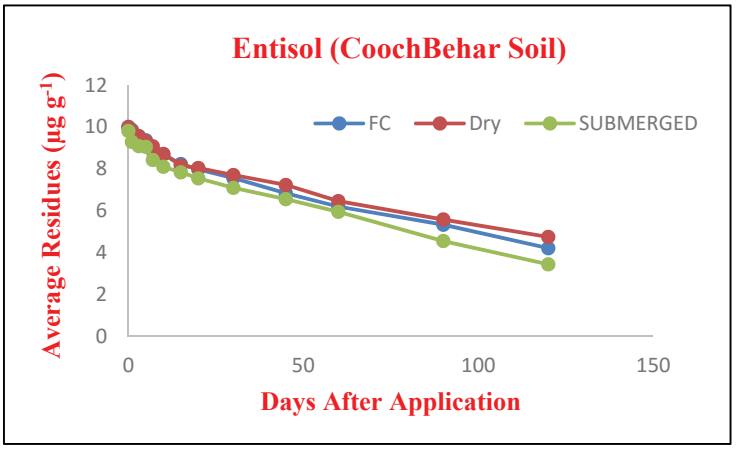


Figure 11E: Persistence of Fluopyram in Entisol under different moisture regimes.

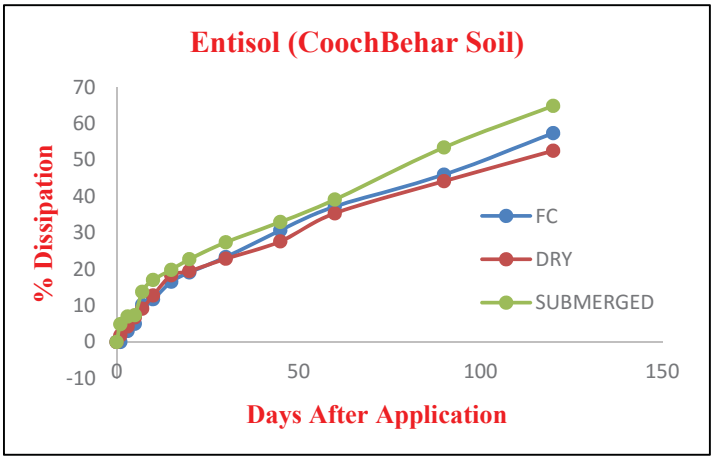


Figure 11F: % dissipation of Fluopyram in Entisol under different moisture regimes

Table (4B): Regression equation for first order dissipation of Flupyradifurone and Fluopyram in Air Dry condition of Entisol :

Chemicals used	Spiking level (ppm)	Regression eq.	R^2	K	Half-life (days)
Flupyradifurone	10	y = -0.0011x + 0.9914	0.99	0.002533	273.63
Fluopyram	10	y = -0.0026x + 0.9756	0.99	0.005900	115.76

In air-dry moisture condition of Alfisol degradation of both the compounds were taken place at a slow rate. The soil sample was fortified at 10 ppm level. At 60, 45, 30, 10 and 5 days fortification, residues of both the compound were detected to be as 8.42, 8.75, 9.12, 9.52, 9.64 ppm and 6.01, 6.70, 7.23, 8.75, 9.15 ppm respectively. Flupyradifurone and fluopyram degradation pattern followed 1st order rate kinetics. Half-life of both the compounds were reported to be as 301 and 107.50 days in Alfisol. Regression equation corresponding to 1st order rate kinetics has been depicted in Table 4C.

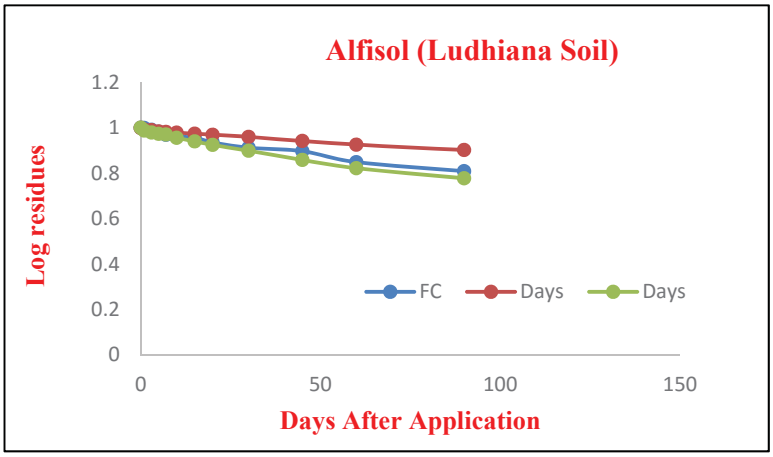


Figure 12A: Persistence of Flupyradifurone in Alfisol under different moisture regimes

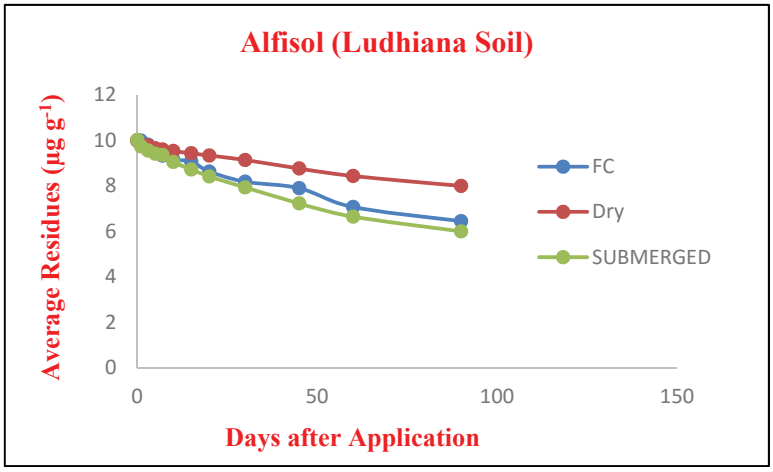


Figure 12B: Persistence of Flupyradifurone in Alfisol under different moisture regimes

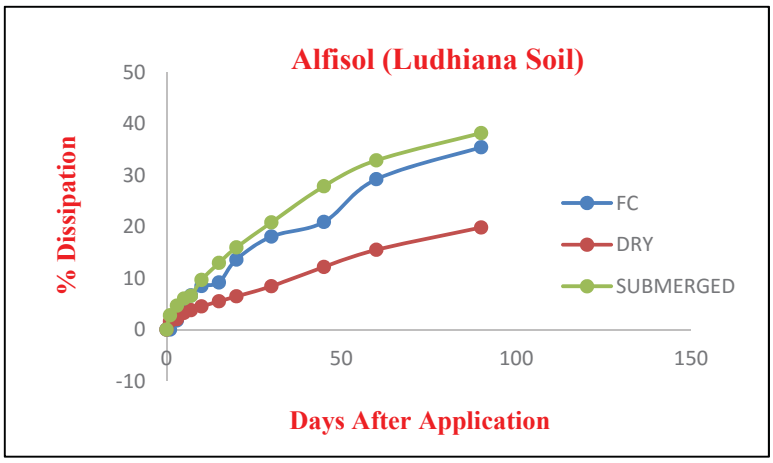


Figure 12C: % dissipation of Flupyradifurone in Alfisol under different moisture regimes

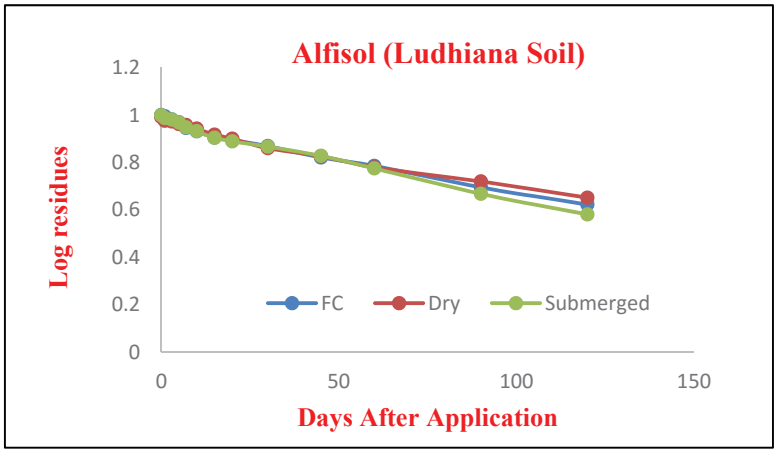


Figure 12D: Persistence of Fluopyram in Alfisol under different moisture regimes

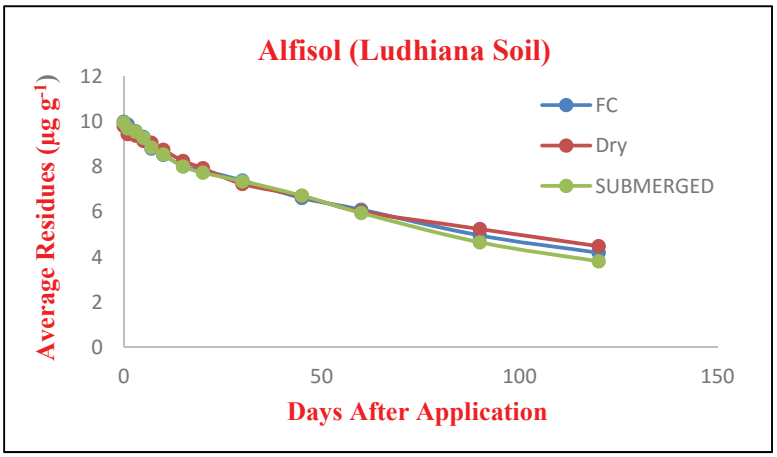


Figure 12E: Persistence of Fluopyram in Alfisol under different moisture regimes

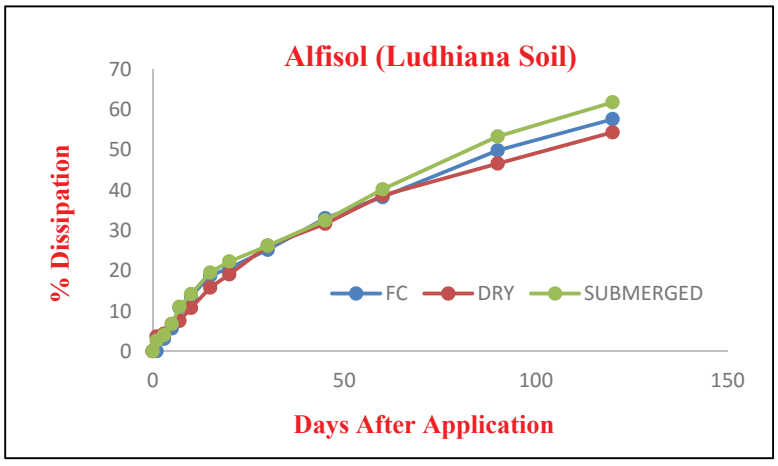


Figure 12F: % dissipation of Fluopyram in Alfisol under different moisture regimes

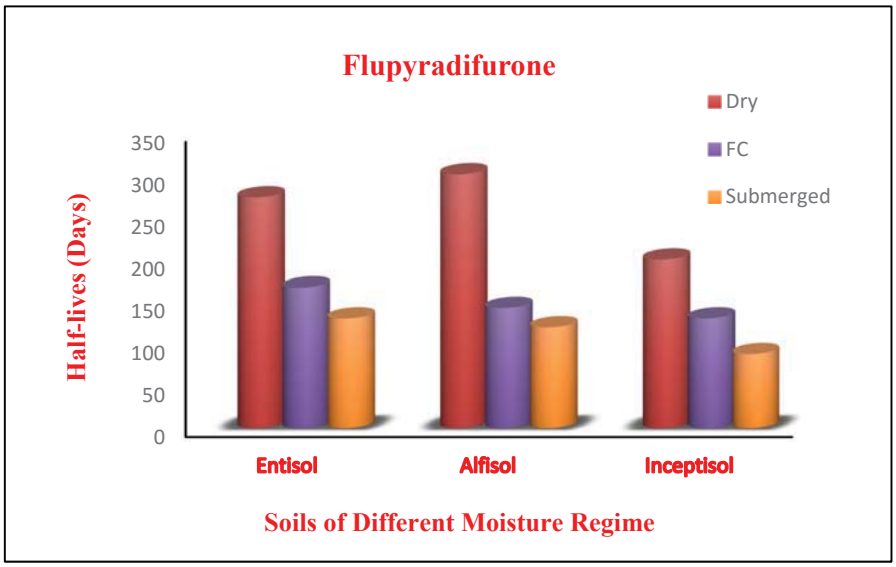


Figure 12G: Half-life of Flupyradifurone in Different Soil Moisture Regime

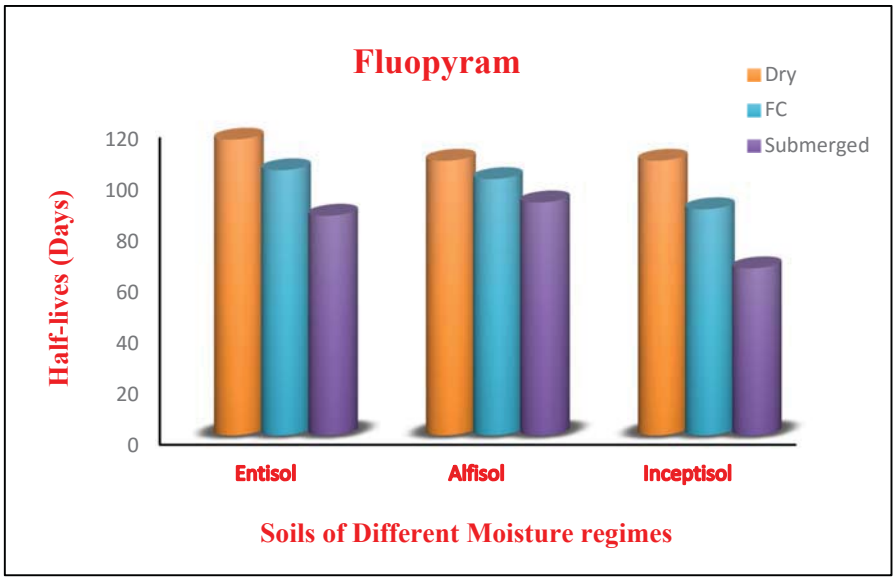


Figure 12H: Half-life of Fluopyram in Different Soil Moisture Regime

Table (4C): Regression equation for first order dissipation of Flupyradifurone and Fluopyram in Air Dry condition of Alfisol :

Chemicals used	Spiking level (ppm)	Regression eq.	R^2	K	Half-life (days)
Flupyradifurone	10	y = -0.0012x + 0.9914	0.98	0.002302	301
Fluopyram	10	y = -0.0028x + 0.9798	0.98	0.006447	107.5

4.3.2 At Field-capacity condition flupyradifurone and fluopyram persistence in soil:

In Table (1, 2, 3, 4, 5 & 6) (Appendix) residue information of Flupyradifurone and Fluopyram has been presented. At FC (field-capacity) condition, up to 120 days, both compound's residue were identified.

In FC (field-capacity) condition of Inceptisol degradation of both the compounds were taken place at a slow rate. The soil sample was fortified at 10 ppm level. At 60, 45, 30, 10 and 5 days fortification, residues of both the compound were detected to be as 6.92, 7.43, 8.14, 9.09, 9.60 ppm and 5.65, 6.52, 7.24, 8.55, 9.32 ppm respectively. Flupyradifurone and fluopyram degradation pattern followed 1^{st} order rate kinetics. Half-life of both the compounds were reported to be as 130.86 and 88.52 days in Inceptisol. Regression equation corresponding to 1^{st} order rate kinetics has been depicted in Table 4D.

Table (4D): Regression equation for first order dissipation of Flupyradifurone and Fluopyram in field-capacity soil of Inceptisol :

Chemicals used	Spiking level (ppm)	Regression eq.	R^2	K	Half life (days)
Flupyradifurone	10	y = -0.0023x + 0.9879	0.98	0.005295	130.86
Fluopyram	10	y = -0.0034x + 0.9726	0.98	0.007828	88.52

Likewise, in Entisol, dissipation rate of flupyradifurone and fluopyram is also slow in FC (field-capacity) condition. The soil sample was fortified at 10 ppm level. At 60, 45, 30, 10 and 5 days fortification, residues of both the compound were detected to be as 7.46, 8.19, 8.44, 9.28, 9.70 ppm and 6.18, 6.82, 7.55, 8.69 and 9.35 ppm respectively. Flupyradifurone and fluopyram degradation pattern followed 1st order rate kinetics. Half-life of both the compounds were reported to be as 167.22 and 103.79 days in Entisol. Regression equation corresponding to 1st order rate kinetics has been depicted in Table 4E.

Table (4E): Regression equation for first order dissipation of Flupyradifurone and Fluopyram in field-capacity condition of Entisol :

Chemicals used	Spiking level (ppm)	Regression eq.	R^2	K	Half life (days)
Flupyradifurone	10	y = -0.0018x + 0.9914	0.99	0.004144	167.22
Fluopyram	10	y = -0.0069x + 0.9707	0.99	0.006677	103.79

In FC (field-capacity) condition of Alfisol degradation of both the compounds were taken place at a slow rate. The soil sample was fortified at 10 ppm level. At 60, 45, 30, 10 and 5 days fortification, residues of both the compound were detected to be as 7.06, 7.89, 8.17, 9.13 and 9.61 ppm and 6.09, 6.61, 7.39, 8.53 and 9.32 ppm respectively. Flupyradifurone and fluopyram degradation pattern followed 1st order rate kinetics. Half-life of both the compounds were reported to be as 143.33 and 100.33 days in Alfisol. Regression equation corresponding to 1st order rate kinetics has been depicted in Table 4F.

Table (4F): Regression equation for first order dissipation of Flupyradifurone and Fluopyram in field-capacity condition of Alfisol :

Chemicals used	Spiking level (ppm)	Regression eq.	R^2	K	Half life (days)
Flupyradifurone	10	y = -0.0021x + 0.9884	0.99	0.004834	143.33
Fluopyram	10	y = -0.003x + 0.9703	0.99	0.006907	100.33

4.3.3 Persistence of Flupyradifurone and Fluopyram in soil under submerged condition:

In Table (1, 2, 3, 4, 5 & 6) (Appendix) residue information of Flupyradifurone and Fluopyram has been presented. At Submerged condition, up to 120 days, both compound's residue were identified.

In submerged condition degradation of both the compound's residues were taken place at a slow rate in Inceptisol. The soil sample was fortified at 10 ppm level. At 60, 45, 30, 10 and 5 days fortification, residues of both the compound were detected to be as 5.65, 6.41, 7.63, 8.78, 9.24 ppm and 4.92, 5.87, 6.89, 8.50 and 9.25 ppm respectively. Flupyradifurone and fluopyram degradation pattern followed 1st order rate kinetics. Half-life of both the compounds were reported to be as 88.50 and 65.43 days in Inceptisol. Regression equation corresponding to 1st order rate kinetics has been depicted in Table 4G**.**

Table (4G): Regression equation for first order dissipation of Flupyradifurone and Fluopyram in submerged condition of Inceptisol :

Chemicals used	Spiking level (ppm)	Regression eq.	R^2	K	Half life (days)
Flupyradifurone	10	y = -0.0034x + 0.9805	0.98	0.007827	88.52
Fluopyram	10	y = -0.0045x + 0.979	0.99	0.010591	65.43

Likewise, in Entisol, dissipation rate of flupyradifurone and fluopyram is also slow in submerged condition. The soil sample was fortified at 10 ppm level. At 60, 45, 30, 10 and 5 days fortification, residues of both the compound were detected to be as 7.02, 7.50, 8.18, 9.26, 9.63 ppm and 5.94, 6.04, 7.08, 8.09, 9.03 ppm respectively. Flupyradifurone and fluopyram degradation pattern followed 1st order rate kinetics. Half-life of both the compounds were reported to be as 130.82 and 86 days in Entisol. Regression equation corresponding to 1st order rate kinetics has been depicted in Table 4H.

Table (4H): Regression equation for first order dissipation of Flupyradifurone and Fluopyram in submerged condition of Entisol :

Chemicals used	Spiking level (ppm)	Regression eq.	R^2	K	Half-life (days)
Flupyradifurone	10	y = -0.0018x + 0.9914	0.99	0.004144	167.22
Fluopyram	10	y = -0.0035x + 0.9635	0.99	0.008058	86.52

In submerged moisture condition of Alfisol degradation of both the compounds were taken place at a slow rate. The soil sample was fortified at 10 ppm level. At 60, 45, 30, 10 and 5 days fortification, residues of both the compound were detected to be as 6.63, 7.21, 7.89, 9.03, 9.40 ppm and 5.95, 6.72, 7.35, 8.54, 9.28 ppm respectively. Flupyradifurone and fluopyram degradation pattern followed 1st order rate kinetics. Half-life of both the compounds were reported to be as 120.40 and 91.21 days in Alfisol. Regression equation corresponding to 1st order rate kinetics has been depicted in Table 4I.

Table (4I): Regression equation for first order dissipation of Flupyradifurone and Fluopyram in submerged condition of Alfisol :

Chemicals used	Spiking level (ppm)	Regression eq.	R^2	K	Half-life (days)
Flupyradifurone	10	y = -0.0021x + 0.9884	0.99	0.004834	143.33
Fluopyram	10	y = -0.0034x + 0.9768	0.99	0.007598	91.21

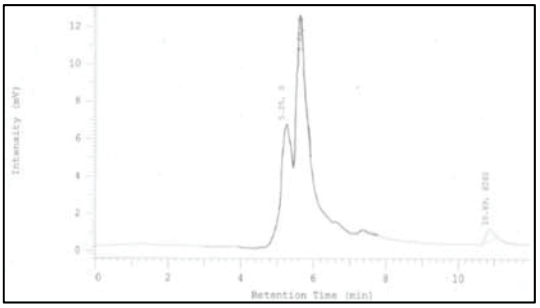


Figure 13A: HPLC chromatogram of Flupyradifurone for persistence 45d

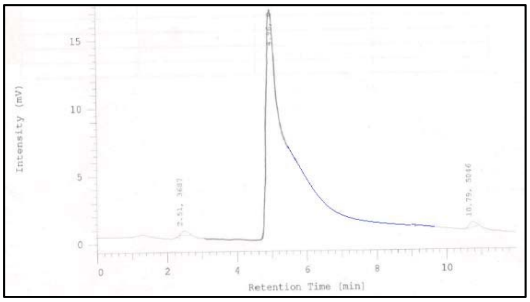


Figure 13B: HPLC chromatogram of Fluopyram for persistence (45d)

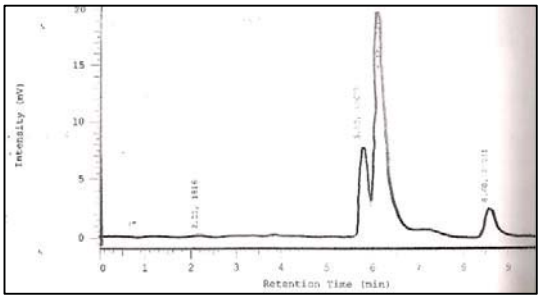


Figure 13C: HPLC chromatogram of Flupyradifurone for persistence 90d

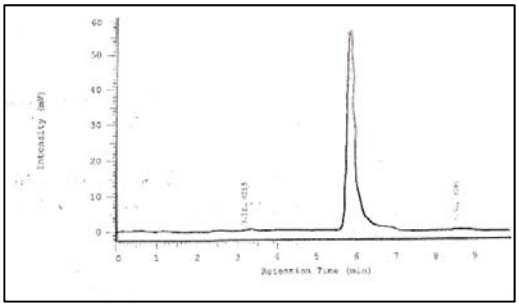


Figure 13D: HPLC chromatogram of Fluopyram for persistence (90d)

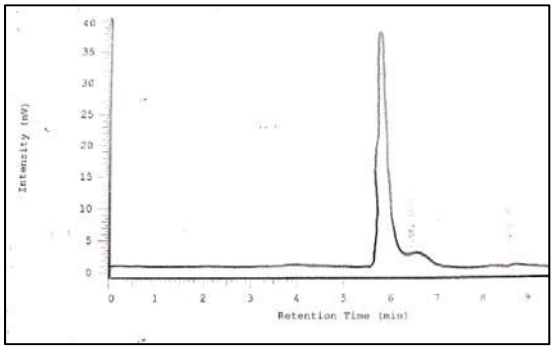


Figure 13E: HPLC chromatogram of Flupyradifurone for persistence 120d

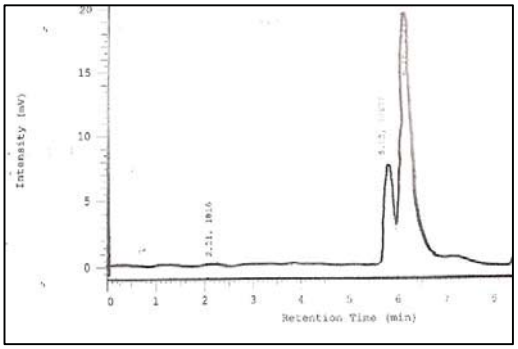


Figure 13F: HPLC chromatogram of Fluopyram for persistence (120d)

4.4 FLUPYRADIFURONE AND FLUOPYRAM PERSISTENCE STUDY IN MANURES:

At 10 ppm level of fortification, persistence of both the compounds were studied in Farm Yard Manure at FC (field-capacity) moisture condition.

4.4.1 Study of Flupyradifurone and Fluopyram persistence at FC (field-capacity) in manure:

In Table [(7 & 8) (Appendix)] residue information of Flupyradifurone and Fluopyram has been presented. At Submerged condition, up to 120 days, both compound's residue were identified.

In FC (field-capacity) condition of Farm Yard Manure degradation of both the compounds were taken place at a slow rate. The soil sample was fortified at 10 ppm level. At 60, 45, 30, 10 and 5 days fortification, residues of both the compound were detected to be as 6.78, 7.50, 8.12, 9.02, 9.45 ppm and 6.90, 7.37, 7.82, 8.78, 9.46 ppm respectively. Flupyradifurone and fluopyram degradation pattern followed 1st order rate kinetics. Half-life of both the compounds were reported to be as 130 and 136 days in Alfisol. Regression equation corresponding to 1st order rate kinetics has been depicted in Table 4J.

Table (4J): Regression equation for first order dissipation of Flupyradifurone and Fluopyram in FYM in field-capacity condition:

Chemicals used	Spiking level (ppm)	Regression eq.	R^2	K	Half-life (days)
Flupyradifurone	10	y = -0.0023x + 0.9846	0.98	0.005295	130.86
Fluopyram	10	y = -0.0022x + 0.9768	0.97	0.005065	136.81

4.5 STUDY OF LEACHING POTENTIALITY OF FLUPYRADIFURONE AND FLUOPYRAM IN DIFFERENT MOISTURE REGIMES OF SOIL

4.5.1 Impact of type of soils on leaching behaviour under water flow (continuous):

Three separate soil types i.e. Inceptisol, Alfisol and Entisol were taken individually for studying leaching behaviour of Flupyradifurone and Fluopyram. Fortification of each soil column was performed at 20 ppm level of flupyradifurone and fluopyram separately. To compare their mobility, 400mL of water (566.10 mm equivalent rainfall) was leached. All the data regarding leaching behaviour of two compounds has been presented in Table 27, 28, 29, 30, 31 and 32 (Appendix).

In three different soil types i.e. Inceptisol, Alfisol, and Entisol, recovery of Flupyradifurone in leachate were 1.08 ppm, 0.22 ppm and 0.09 ppm respectively from 20 ppm residue. In Inceptisol residues of Flupyradifurone and Fluopyram were 0.71, 1.21, 15.49 ppm and 0.43, 1.29, 14.53 ppm individually at a soil depth of 10-15, 5-10, 0-5 cm and none of the compounds were detected beyond 15cm depth of soil. In Alfisol, flupyradifurone residues were 1.13, 1.40 ppm and fluopyram residues were 1.01, 13.55 ppm at 5-10 and 0-5 cm depth of soil and none of the compounds could be identified beyond the soil depth of 10 cm. In the case of Entisol, detection limit for the residues in the soil column is up to 5 cm. Residues of flupyradifurone at 0-5 cm depth were 10.11 ppm and residues of fluopyram were 9.59 ppm.

Highest leaching potentiality was observed in Inceptisol followed by Alfisol and minimum in Entisol due to presence of high amount of sand content in Inceptisol as compared to Alfisol and Entisol.

Table (5A): Mobility behaviour of Flupyradifurone in soils under continuous flow conditions

Water volume (mL)	Simulating rainfall (mm)	Treatment replications	Residues in matrix used (Soil) (ppm) Depth of column (cm)					Residues in Leachate (ppm)
			0-5	5-10	10-15	15-20	20-25	
Flupyradifurone in Inceptisol								
400	566	R1	15.47	1.32	0.52	BQL	ND	1.08
		R2	15.51	1.10	0.89	BQL	ND	
		Mean	15.49	1.21	0.71	BQL	ND	
		% of recovered	77.42	6.09	3.56	BQL	ND	
Flupyradifurone in Alfisol								
400	566	R1	14.62	1.17	BQL	BQL	ND	0.22
		R2	14.37	1.10	BQL	BQL	ND	
		Mean	14.50	1.13	BQL	BQL	ND	
		% of recovered	72.50	5.69	BQL	BQL	ND	
Flupyradifurone in Entisol								
400	566	R1	10.34	BQL	BQL	BQL	ND	0.09
		R2	9.88	BQL	BQL	BQL	ND	
		Mean	10.11	BQL	BQL	BQL	ND	
		% of recovered	50.57	BQL	BQL	BQL	ND	

BQL (< 0.05 µg g^{-1})

Table (5B): Mobility behaviour of Fluopyram in soils under continuous flow conditions

Water volume (mL)	Simulating rainfall (mm)	Treatment replications	Residues in matrix used (Soil) (ppm) Depth of column (cm)					Residues in Leachate (ppm)
			0-5	5-10	10-15	15-20	20-25	
Fluopyram in Inceptisol								
400	556	R1	14.47	1.27	0.45	BQL	ND	0.94
		R2	14.59	1.31	0.41	BQL	ND	
		Mean	14.53	1.29	0.43	BQL	ND	
		% of recovered	72.67	6.47	2.17	BQL	ND	
Fluopyram in Alfisol								
400	556	R1	13.68	0.98	BQL	BQL	ND	0.10
		R2	13.42	1.04	BQL	BQL	ND	
		Mean	13.55	1.01	BQL	BQL	ND	
		% of recovered	67.82	5.06	BQL	BQL	ND	
Fluopyram in Entisol								
400	556	R1	7.89	BQL	BQL	BQL	ND	0.08
		R2	8.28	BQL	BQL	BQL	ND	
		Mean	8.09	BQL	BQL	BQL	ND	
		% of recovered	40.44	BQL	BQL	BQL	ND	

BQL (< 0.05 µg g^{-1})

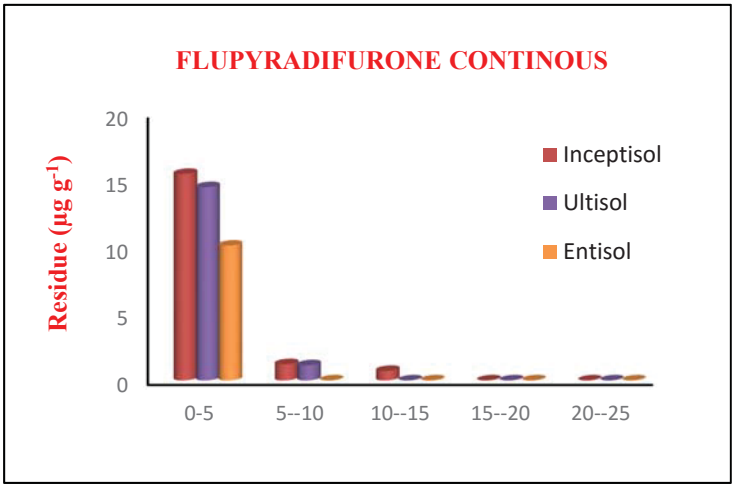


Figure 16A: Residues of flupyradifurone detected in soil column under continuous condition

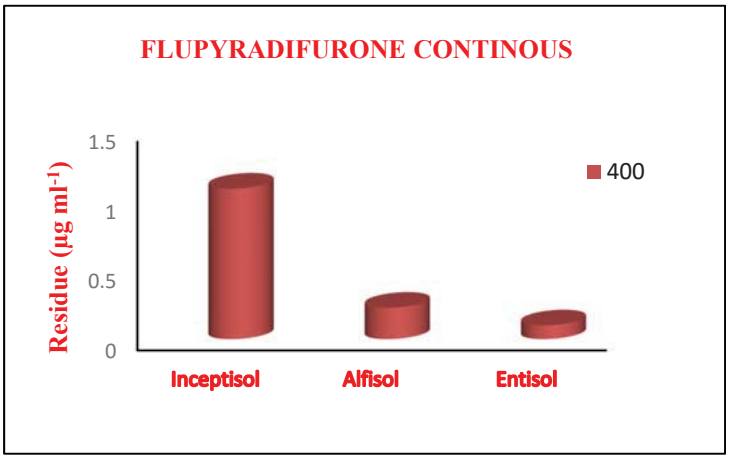


Figure 16B: Residues of Flupyradifurone detected in leachate under continuous flow condition

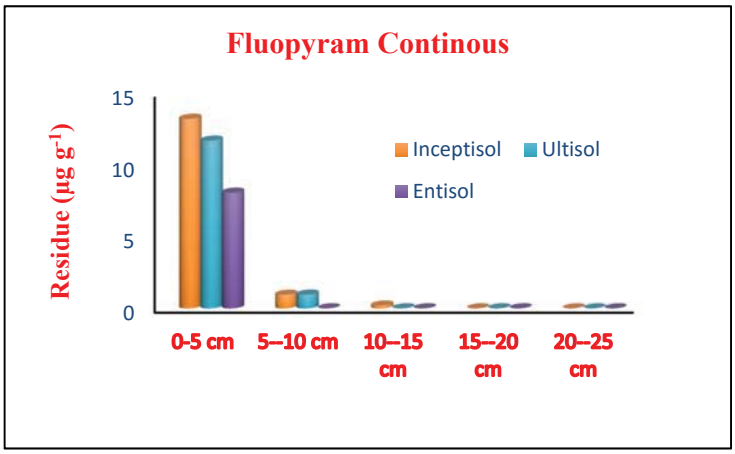


Figure 16C: Residues of Fluopyram detected in soil column under continuous condition

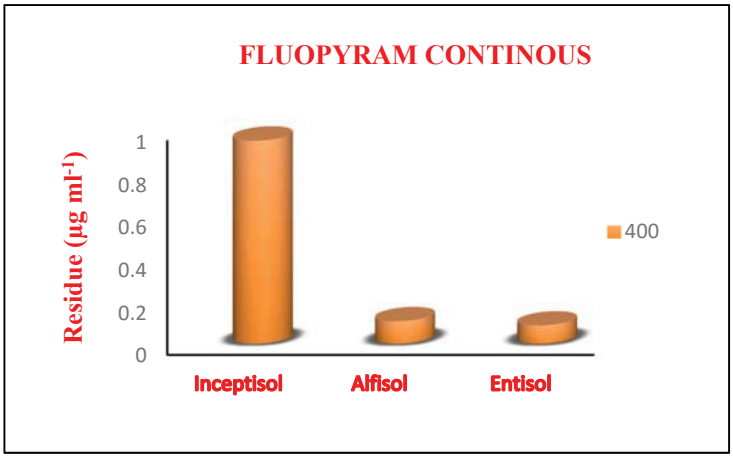


Figure 16D: Residues of Fluopyram detected in leachate under continuous flow condition

4.5.2 Influence of types of soil on mobility behaviours of Flupyradifurone and Fluopyram on varying amounts of water:

Three polythene columns were filled with three distinct type of soil and fortification were done with flupyradifurone and fluopyram separately at 20 ppm level. The columns with varying amount of water i.e. 40ml (56.61mm equivalent rainfall), 80ml (113.23mm equivalent rainfall), 160ml (226.46 mm equivalent rainfall) and 240ml (340 mm equivalent rainfall) were leached. Collection and analysis of each fraction of separate soil columns has been done. It was seen that with increase in water amount, both the compounds' mobility was also increased in soil column.

In the Table 21, 22, 23, 24, 25 & 26 (Appendix), leaching findings for three distinct soils under discontinuous water flow conditions of two compounds are demonstrated.

On leaching of the soil column with 40 ml of water, residues of both the compounds were found to be at 5-10 cm depth of soil. However, the compounds had moved to lower depth of the column when the water quantity was increased.

In Inceptisol, at 0-5 cm depth of soil, obtainable residues for both the compound were highest (13.89 and 12.22 ppm), In case of Alfisol, the amounts are 10.90 and 10.70 ppm respectively and for Entisol, the values are 8.70 and 8.08 ppm. Residues are leached to lower depth of column on addition of more quantity of water. For Inceptisol, Flupyradifurone and Fluopyram were identified up to a depth of 10-15

cm, in case of Alfisol, 5-10 cm is the soil depth at which both the compounds were identified and for Entisol, at 5 cm soil depth only, identification of both the compounds were possible. In Inceptisol residues of both the compound were identified in greater soil depth compared to Alfisol and Entisol. Both the compound's residue detected in leachate under discontinuous condition were 0.58, 036, 0.17, 0.07 ppm and 0.47, 0.25, 0.10, 0.05 ppm from 240, 160, 80, 40 ml in Inceptisol , 0.12, 0.06 ppm and 0.05 ppm from 240 and 160 ml in Alfisol and 0.05 ppm and 0.05 ppm from 240 ml in Entisol , respectively.

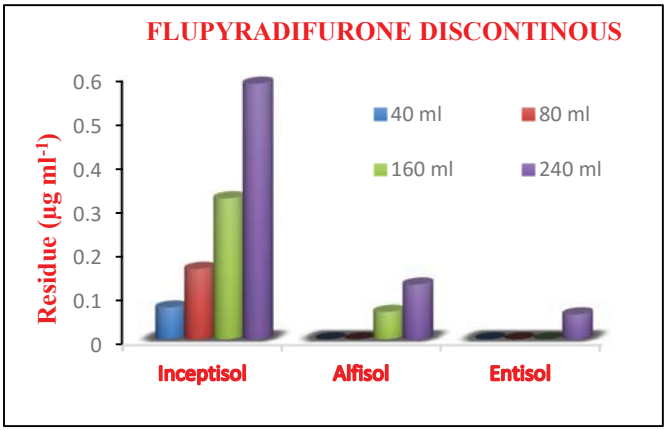


Figure 17A: Residues of Flupyradifurone detected in soil column under discontinuous condition

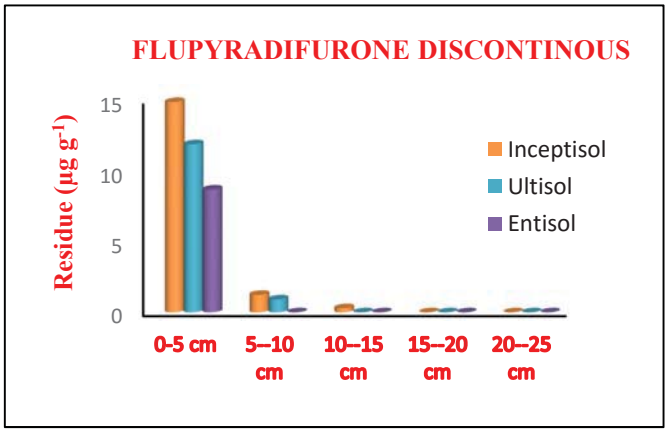


Figure 17B: Residues of flupyradifurone detected in leachate under discontinuous flow condition

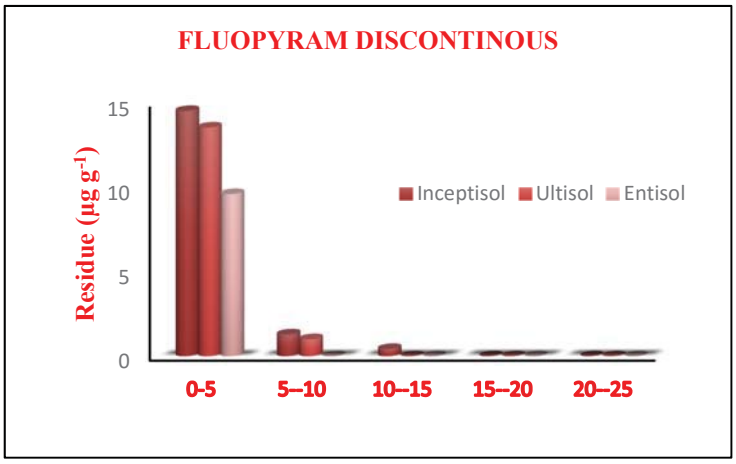


Figure 17C: Residues of Fluopyram detected in soil column under discontinuous condition

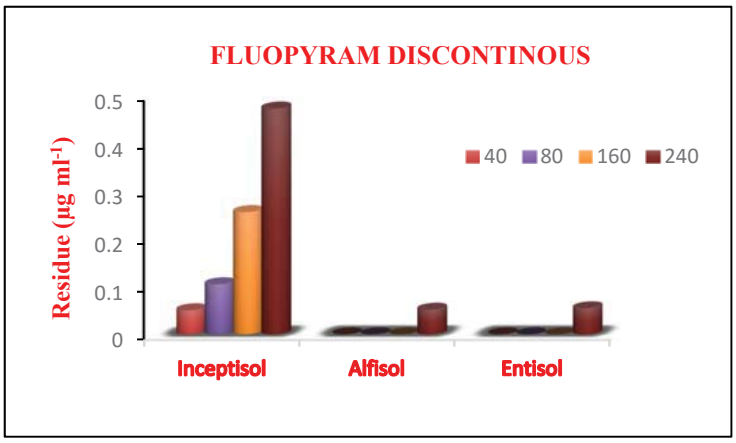


Figure 17D: Residues of Fluopyram detected in leachate under discontinuous flow condition

Chapter 5

DISCUSSION

Flupyradifurone and fluopyram were analyzed using High Performance Liquid Chromatography. In order to achieve accurate resolution and precise determination of the test compounds from soil and water samples, the parameters of HPLC were standardized individually. UV-VIS detector was linked to the instrument to demonstrate the distinct resolution for both the compound. The finest solvent system for estimation of flupyradifurone and fluopyram has been determined as acetonitrile: water (70:30, v /v), with a retention time of 5.8 minute and 6.4 minute. 0.1 per cent Formic Acid in 95 per cent acetonitrile-water was another mobile phase used in the experiment. However, the minimum identified concentration was recorded to show the highest resolution, sensitivity and linearity. The detection limits (LOD) were 0.005 ppm for both flupyradifurone and fluopyram. Limit of Quantification (LOQ) for flupyradifurone and fluopyram has been noted as a minimum quantifiable limit for both compounds as 0.05 ppm.

Liquid-Liquid partitioning is used to extract both the compounds from aqueous samples. Dichloromethane was proved to be the best solvent for extraction of the compounds among dichloromethane and ethyl acetate. Methods, assessed to determine the best technique for extracting both the compounds from soil samples were modified QuEChERS, single step extraction and dipping & shaking. Dipping and shaking proved to be an effective extraction technique among others followed by modified QuEChERS and single step extraction. Despite the reality that the QuEChERS technique is a quicker method and extricate solvent amount and time, the method is more costly compared to rest 2 procedures, so the Dipping and shaking technique was selected for this experiment.

It has been noted that degradation of flupyradifurone and fluopyram with respect to moisture regimes and type of soil was slow in three distinct kinds of soil. As because of high microbial activity and elevated organic matter content, Ultisol was more rapid in the persistence research of flupyradifurone and fluopyram dissipation as compared to Entisol and Inceptisol. Flupyradifurone persistence in all three soil types was greater than fluopyram.

The pattern of dissipation for both the compounds followed 1st order rate kinetics as conditions of environment such as microbial activity, temperature, humidity and compound molecule share in free and adsorbed form were retained in steady circumstances throughout the entire experimental era. Under three distinct conditions of moisture, such as Submerged, FC (field-capacity) and air dry, dissipation of both the compounds were examined. In the three conditions of moisture, maximum flupyradifurone and fluopyram dissipation were noted in Submerged Soil, followed by FC and Air dry moisture condition. In Dry condition of Inceptisol, elementary residues of flupyradifurone & fluopyram were acquired after 1 day level of fortification as 9.79 ppm & 9.67 ppm and after fortification of 120 days, the residues obtained were 7.34 ppm & 5.47 ppm. In Inceptisol, at FC moisture regime, dissipation of residues were taken place from 9.94 (1d) to 6.92 ppm (120d) for flupyradifurone and from 9.83 (1d) to 4.89 ppm (120d) for fluopyram. In Submerged condition of Inceptisol, dissipation pattern was observed as 9.65 (1d) to 5.06 ppm (120d) for flupyradifurone and 9.58 (1d) to 3.82 ppm (120d) for Fluopyram. Similarly, for Entisol residues of both the compounds dissipated in air-dry condition from 9.88 (1d) to 7.97 ppm (120d) for Flupyradifurone and 9.80 (1d) and 5.87 ppm (120d) for fluopyram. At FC moisture regime of Entisol, dissipation pattern of dissipation were found to be as 9.89 (1d) to 6.92 ppm (120) for Flupyradifurone and 9.85 (1d) to 5.45 ppm (120d) for Fluopyram. In Entisol, under Submerged condition, the residues of both the compounds were dissipated from 9.84 (1d) to 6.37 (120d) ppm for Flupyradifurone and 9.28 (1d) to 4.54 ppm (120d) for Fluopyram. Similar dissipation rate was also noted in case of Ultisol . Both the compounds dissipated in air-dry condition from 9.81 (1d) to 7.98 ppm (120d) for Flupyradifurone and from 9.45 (1d) to 4.24 ppm (120d) in case of Fluopyram. In FC moisture regime, values were 9.98 (1d) to 6.44 ppm (120d) for Flupyradifurone and 9.88 (1d) to 4.95 ppm (120d) for Fluopyram. In Submerged condition, rate of dissipation was 9.73 (1d) to 5.99 ppm (120d) for Flupyradifurone and 9.70 (1d) to 4.65 ppm (90d) for Fluopyram, respectively. In air-dry condition half-life of Flupyradifurone in Ultisol , Entisol and Inceptisol are 301, 273.63 & 200 days. 167.22, 143.33 & 130.86 days for FC moisture regime and 130.22, 120.4 & 88.52 days for Submerged condition, respectively. In Ultisol , Entisol and Inceptisol half-lives of Fluopyram are 115.76, 110.7 and 107.5 days for air-dry condition, 103.79, 100.33 & 88.50 days for FC moisture regime and 91.22, 86 & 65.43 days for Submerged condition, respectively.

In submerged condition, pattern of dissipation for both the compounds were identified to be rapid, hence persist for longer time due to presence of ideal anaerobic microbial community followed by FC moisture regime and minimum in air dry condition.

It was found that the dissipation rate of Flupyradifurone & Fluopyram in manure was steady. In Farm Yard Manure half-life of flupyradifurone and fluopyram were recorded to be as 130.86 and 136.4 days at FC moisture regime. Residues of both the compounds were dissipated from 9.98 (1d) to 6.22 ppm (120d) for flupyradifurone and from 9.88(1d) to 6.44 ppm (120d) for Fluopyram.

As stated by the existence of aromatic amide ring on the fluopyram, fluopyram showed greater persistence compared to flupyradifurone owing to distinct molecular structure. Because of the fluorine atom's negative charge, fluopyram binds with soil particles as adsorbed by clay and persists for a long time. On the other hand, flupyradifurone, comprised of a pyridinyl ring which may not be as strongly adsorbed to clay as fluopyram. Therefore, fluopyram adsorption capability is greater in the soil and therefore persistence is greater.

In three columns filled with three different types of soil, Leaching Experiment was conducted with Flupyradifurone and Fluopyram. On increasing amount of water from 40 ml to 240 ml, both compound's mobility slowly increases and residues shifting of both the compound's residues takes place from the top to bottom of the column. The entire leaching research performed with flupyradifurone and fluopyram in Inceptisol, Ultisol and Entisol showed higher mobility of the compounds in Inceptisol than two other type of soil. Below 10 cm residues of both the compound could not be identified in Ultisol and for Entisol detection of both the compounds were not possible below 5 cm soil depth. As Inceptisol carries a large quantity of sand which helps to transfer the compounds to a depth of lower height, similar outcome was also noted in case of leachate. In Ultisol organic matter content and clay percentage is high and due to the presence of elevated sodium salt, both the compounds were completely dispersed in Entisol and their movement were reducing through the soil column. More leaching was noted under constant water flow condition compared to Discontinuous water flow condition. The highest recovery of flupyradifurone from soil in continuous flow was 77.47 percent at 0-5 cm depth in Inceptisol, where 72.50 percent at 0-5 cm depth in Ultisol and 50.57 percent at 0-5 cm depth in Entisol, but in the case of fluopyram recovery of 72.67 percent, 67.78 percent

and 47.99 percent were identified at 0-5 cm depth in Inceptisol, Ultisol & Entisol separately. As both the compounds were highly sorbed at the upper part of the soil column, movement of the compounds were restricted at the lower part. Inceptisol displayed maximum leaching capacity followed by Ultisol & minimum in Entisol. Flupyradifurone has more Leaching capacity in comparison to fluopyram owing to its greater solubility, which can be concluded after a complete Leaching experiment.

Chapter 6

SUMMARY AND CONCLUSION

The use of neonicotinoids in integrated pest management (IPM) programs has been highly appreciated by growers. They provide a distinctive mode of action to handle insecticide-resistant pests. Neonicotinoids regulate insect pests selectively while ensuring that beneficial insects are accessible to manage other potential insect pests. AgInfomatics reported “neonicotinoid insecticides provide an average increase in the yield varying from 3.6 to 71.3 per cent in eight main plant habitats in North America throughout an extensive financial assessment of over 1500 field study”. This study has shown that the average return of neonicotinoids exceeds the treatment costs far and gives the farmer a significant economic profit.

Flupyradifurone, a new neonicotinoid insecticide developed by Bayer CropScience under the trademark "SivantoTM," licensed and worldwide marketable, is regarded persistent to very persistent in major climatic areas of the globe and can be regarded as moderately mobile to mobile depending on soil circumstances. It, therefore, can reach aquatic environments, including surface and groundwater, for several months or longer after implementation (Chawla *et al*.,2018). It is non-volatile in nature and therefore air movement is not going to be a significant transport route for the compound. Accessible fate data implies that flupyradifurone is likely to dissipate from point of use through several transport processes including runoff, erosion, and leaching of groundwater. Although the pesticide mass (DT_{90}) reduction was often more than one year in field dissipation studies in the Terrestrial Field Research.

Fluopyram, a pyridylethylamide compound invented by Bayer CropScience and launched as a broad range fungicide in April 2012 under the trade name Luna Privilege. Used in numerous horticultural and arable crops (outdoor strawberries, peas, apples, indoor and outdoor lettuces, pears, indoor peppers and beans) to safeguard against a variety of Deuteromycetes and ascomycetes. Very few studies have been conducted to illustrate the pattern and risk assessment of fluopyram dissipation when used as a foliage applied fungicide (Chawla *et al*., 2018).

The thesis presents, a study entitled **"Persistence and Mobility of Flupyradifurone and Fluopyram in soils with different moisture regimes"** was carried out in Laboratory conditions.

Following are the important characteristics of the experiment under laboratory circumstances:

Based on research of recovery, Clean-up and Extraction techniques were standardized for the assessment of Flupyradifurone and Fluopyram separately from water and soil samples. The HPLC parameters for Flupyradifurone and Fluopyram were optimized and standardized individually. Acetonitrile: water (70:30, v / v) is the solvent system, optimized for both of the compounds. Entire analysis was carried out at 280 nm wavelength (λ_{max}). Samples of water spiked at 10 ppm level. Greater proportion of recovery from samples of water was obtained by using DCM (95.8%) as compared to Ethyl Acetate (89.1%) for Flupyradifurone and 95.5% & 86.1% recovery was obtained from DCM (dichloromethane) and EtOAc (ethyl acetate) in case of fluopyram. For extraction, from soil samples, three distinct methods such as dipping and shaking, QuEChERS technique and single step extraction technique were applied. Highest recovery of 95% for Flupyradifurone and 92.4% for Fluopyram was obtained by using dipping and shaking method. The dipping and shaking technique was therefore chosen to perform the entire experiments. For both the compounds, Flupyradifurone & Fluopyram, Limit of Detection (LOD) were 0.005 ppm and Limit of Quantification (LOQ) were 0.05 ppm.

Persistence experiments of both the compounds were performed under three moisture regimes, such as, submerged, FC (field-capacity) and air-dry condition. The persistence research discovered that flupyradifurone dissipated more quickly in submerged condition ($t_{1/2}$ 88.50, 120.45 and 130.81 days) followed by FC moisture regime ($t_{1/2}$ 130.85, 143.34 and 167.22 days) and air-dry condition ($t_{1/2}$ 100, 273.67 and 301 days) respectively in Inceptisol, Ultisol and Entisol . In the case of fluopyram similar results has also been observed. The half-life ($T_{1/2}$ in days) of the fluopyram under submerged condition was 65.4, 91.2 and 86 days followed by 103.7, 100.0 and 89.5, 101 and 103.7 days in FC moisture regime and 102, 115.7 and 107.5 days in dry condition in Inceptisol, Ultisol and Entisol respectively. The outcome indicates that flupyradifurone and fluopyram both are more common in air-dry moisture condition and less obstinate in submerged circumstances. Dissipation of flupyradifurone and

fluopyram was also noted to vary with different soil moisture regimes. Due to presence of high microbial activity and elevated organic matter content, dissipation in Ultisol and Entisol was quicker for both the compounds and less so for Inceptisol . Only under field capability condition, persistence research was performed in FYM manure. Half-lives were 130.8 for flupyradifurone and 136.6 for fluopyram in FYM field-capacity circumstances.

leaching study of Flupyradifurone and Fluopyram was carried out in polythene columns filled with three different types of soil, under constant (400 mL, equivalent to 566.10 mm of rainfall) and discontinuous condition (160, 120, 80 and 40 mL, equivalent to 339.70, 226.46, 113.23 and 56.61 mm of rainfall). In Inceptisol, proportion of residues of both the compounds obtained in leachate, under constant flow was 1.08 ppm and 0.94 ppm, respectively, followed by 0.21 ug g^{-1} and 0.10 ppm in Ultisol and minimum amount is obtained in Entisol i.e. 0.09 ppm and 0.08 ppm respectively. High mobility of both the compounds were observed in Inceptisol, moderate in Ultisol and least in Entisol. The mobility of flupyradifurone and fluopyram reduces according to the depth of soils from 0-5 cm to 20-25 cm due to elevated soil sorption of flupyradifurone and fluopyram. Due to the sandy loam nature of the Inceptisol, both compound's residues were identified up to a depth of 10-15 cm, suggesting elevated mobility of the compounds. However, residues of flupyradifurone and fluopyram were observed in Ultisol up to a soil depth of 5-10 cm, and residues of flupyradifurone and fluopyram in Entisol were below the quantifiable limit (< 0.05 ppm) at below 5 cm soil depth.

ABSTRACT

Persistence and Mobility of Flupyradifurone and Fluopyram in Soils with Different Moisture Regimes

In distinct soils and manure under Indian subtropical circumstances, environmental fate research and dynamics of flupyradifurone and fluopyram are not currently noted. Keeping this information in view, a research entitled **"Persistence and mobility of Flupyradifurone and Fluopyram in soils with different moisture regimes"** was conducted under research laboratory circumstances. Leaching and Persistence studies of flupyradifurone and fluopyram were performed under laboratory circumstances as affected by various variables, such as, primarily water content, physical and chemical characteristics of soil and manure. Both the compounds being studied from soil and water samples were standardized using analytical methods. Dipping and shaking technique followed by partitioning into dichloromethane and subsequently washing the column was discovered to be most appropriate. and parameters of HPLC were standardized discretely for respective compounds. Detection limit (LOD) for both Flupyradifurone and Fluopyram were found to be as 0.005 ppm. Quantification limit (LOQ) for Flupyradifurone and Fluopyram in soil and water samples were noted to be as 0.05 ppm.

In Inceptisol, Ultisol and Entisol, at 10 ppm level of fortification, under three distinct moisture regimes (In case of FYM, only FC moisture regime), relative analysis of Flupyradifurone and Fluopyram persistence was performed. Mobility of both the compounds were studied only in three diverse type of soils at 10 ppm level of fortification. Residues stayed in all persistence study treatments for over 120 days. The values of half-life fluctuated from ($t_{1/2}$) 301, 273.63, 200, 130.33 and 101 days under different moisture regimes for flupyradifurone and ($t_{1/2}$) 115.76, 107.80, 103.79, and 88.52 days for Fluopyram in Ultisol, Entisol, Inceptisol and FYM, respectively. The results revealed that flupyradifurone and fluopyram have highest persistence in Ultisol in aerobic conditions followed by Entisol, Inceptisol, and minimum persistent was observed in FYM under anaerobic conditions.

Leaching studies showed that the mobility of flupyradifurone and fluopyram was heavily affected by soil types and the quantity of organic matter existing. 5.38% and 4.72% of residues of flupyradifurone and fluopyram, respectively were present in leachate in Inceptisol (IARI soil), 1.15% and 0.52% in Ultisol and 0.45% and 0.42% in Entisol (CoochBehar soil), respectively under continuous flow conditions. Flupyradifurone and fluopyram showed maximum mobility in Inceptisol (IARI soil), followed by mild mobility in Ultisol and least in Entisol and flupyradifurone shows greater mobility than fluopyram between two compounds.

Keywords : Flupyradifurone, Fluopyram, Persistence, Mobility, Residues

विभिन्न नमी नियमों के साथ मिट्टी में फ्लुप्रैडिफ्यूरोन और फ्लुपीरम की दृढ़ता और गतिशीलता

भारतीय उपोष्णकटिबंधीय परिस्थितियों में अलग-अलग मिट्टी और खाद में, पर्यावरणीय भाग्य अनुसंधान और फ्लुपीरिड्यूरोन और फ्लुपीरम की गतिशीलता वर्तमान में नोट नहीं की गई है। इस जानकारी को ध्यान में रखते हुए, अनुसंधान प्रयोगशाला परिस्थितियों में "नमी और फ्लुपीरेडिफेरोन और फ्लुपीरियम की गतिशीलता के साथ मिट्टी में विभिन्न नमी शासन के साथ" नामक एक अनुसंधान आयोजित किया गया था। फ्लुप्रैडिफ्यूरोन और फ्लुपीरम के लीचिंग और दृढ़ता अध्ययनों को विभिन्न परिस्थितियों जैसे कि मुख्य रूप से जल सामग्री, मिट्टी और खाद की भौतिक और रासायनिक विशेषताओं से प्रभावित प्रयोगशाला परिस्थितियों में किया गया था। मिट्टी और पानी के नमूनों से अध्ययन किए जा रहे दोनों यौगिकों को विश्लेषणात्मक तरीकों का उपयोग करके मानकीकृत किया गया था। डाइक्लोरोमेथेन में विभाजन के बाद डिपिंग और हिलाने की तकनीक और बाद में कॉलम को धोना सबसे उपयुक्त पाया गया। और एचपीएलसी के मापदंडों को संबंधित यौगिकों के लिए विवेकपूर्ण रूप से मानकीकृत किया गया था। फ्लुप्रैडिफ्यूरोन और फ्लुपीरम दोनों के लिए जांच सीमा (LOD) 0.005 ppm के रूप में पाई गई। मिट्टी और पानी के नमूनों में फ्लुपीरिड्यूरोन और फ्लुपीरम के लिए मात्रा की सीमा (LOQ) 0.05 ppm के रूप में नोट किया गया था।

Inceptisol में, Ultisol और Entisol, किलेबंदी के 10 ppm स्तर पर, तीन अलग-अलग नमी शासन के तहत (FYM के मामले में, केवल FC नमी शासन), फ्लुप्रैडिफ्यूरोन और फ्लुओपैमम दृढ़ता के सापेक्ष विश्लेषण किया गया था। किलेबंदी के 10 पीपीएम स्तर पर दोनों यौगिकों की गतिशीलता का अध्ययन केवल तीन विविध प्रकार की मिट्टी में किया गया था। अवशेष 120 दिनों के लिए सभी दृढ़ता अध्ययन उपचार में रहे। फ्लुप्रैडिफ्यूरोन और ($t_{1/2}$) 115.76, 107.80, 103.79 और Ultisol में फ्लुओपिरम के लिए 88.52 दिनों के लिए अलग-अलग नमी शासन के तहत ($t_{1/2}$) 301, 273.63, 200, 130.33 और 101 दिनों से उतार-चढ़ाव वाले जीवन के मूल्यों , इनसेप्टिसोल और FYM, क्रमशः। परिणामों से पता चला है कि फ्लुपीराडिफुरोन और फ्लुओपिरम में एरोबिक स्थितियों में अल्टिसोल में सबसे अधिक दृढ़ता है, इसके बाद एंटिसोल, इनसेप्टिसोल और एरोबिक स्थितियों के तहत FYM में न्यूनतम दृढ़ता देखी गई।

लीचिंग अध्ययनों से पता चला है कि फ्लुपीराइडिफुरोन और फ्लुओपिरम की गतिशीलता मिट्टी के प्रकारों और कार्बनिक पदार्थों की मात्रा से काफी प्रभावित थी। फ्लुप्रायडिफ्यूरोन और फ्लुपीरम

के अवशेषों का 5.38% और 4.72% क्रमशः इंसेफिसोल (IARI मिट्टी) में लीकेज में, 1.15% और अल्टिसोल में 0.52% और एंटिसोल (कूचबिहार मिट्टी) में क्रमशः 0.42%, निरंतर प्रवाह की स्थिति में मौजूद थे। फ्लुप्रैडिफ्यूरोन और फ्लुपीरम ने Inceptisol (IARI मिट्टी) में अधिकतम गतिशीलता दिखाई, इसके बाद Ultisol में हल्की गतिशीलता और Entisol में कम से कम और फ्लुप्रैडिफ्यूरोन दो यौगिकों के बीच फ्लुओपिरम से अधिक गतिशीलता दिखाता है।

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Table 1: Persistence of Flupyradifurone in Inceptisol (IARI soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Replicates	Residues (µg g^{-1})		
		Inceptisol (IARI soil)		
		Air Dry	Field capacity	Submerged
0	R1	9.97	10.01	9.94
	R2	9.95	10.02	9.99
	Mean ± SD	9.96±0.312	10.01±0.000	9.91±0.103
3	R1	9.86	9.94	9.62
	R2	9.82	9.91	9.69
	Mean ± SD	9.84±0.117	9.92±0.020	9.64±0.121
5	R1	9.76	9.87	8.84
	R2	9.72	9.81	8.80
	Mean ± SD	9.74±0.026	9.84±0.040	8.82±0.048
7	R1	9.53	9.67	7.94
	R2	9.64	9.61	7.92
	Mean ± SD	9.59±0.077	9.64±0.041	7.93±0.016
10	R1	9.34	8.91	7.36
	R2	9.44	8.99	7.21
	Mean ± SD	9.39±0.073	8.96±0.210	7.29±0.101
15	R1	9.18	8.84	7.13
	R2	9.13	8.81	7.09
	Mean ± SD	9.15±0.108	8.82±0.025	7.11±0.0413
20	R1	9.07	8.41	6.97
	R2	9.06	8.48	6.92
	Mean ± SD	9.05±0.133	8.44±0.191	6.95±0.039
30	R1	8.86	8.27	6.83
	R2	8.83	8.21	6.89
	Mean ± SD	8.84±0.046	8.24±0.187	6.96±0.051
45	R1	8.59	7.87	6.76
	R2	8.45	7.87	6.72
	Mean ± SD	8.52±0.097	7.84±0.144	6.74±0.112
60	R1	7.29	6.68	5.84
	R2	7.38	6.64	5.81
	Mean ± SD	7.74±0.067	6.66±0.032	5.82±0.0473
90	R1	7.74	6.34	5.53
	R2	7.80	6.49	5.59
	Mean ± SD	7.37±0.043	6.42±0.114	5.56±0.039
120	R1	6.54	5.02	4.45
	R2	6.56	5.12	4.49
	Mean ± SD	6.55±0.008	5.08±0.128	4.46±0.004

Table 2: Persistence of Fluopyram in Inceptisol (IARI soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Replicates	Residues (µg g^{-1})		
		Inceptisol (IARI soil)		
		Air Dry	Field capacity	Submerged
0	R1	9.99	9.72	10.18
	R2	9.94	9.74	10.26
	Mean ± SD	9.97± 0.034	9.73± 0.012	10.23± 0.050
3	R1	9.75	9.46	9.23
	R2	9.72	9.572	9.40
	Mean ± SD	9.73± 0.115	9.52± 0.078	9.32± 0.119
5	R1	9.58	9.26	8.53
	R2	9.54	9.04	8.61
	Mean ± SD	9.56± 0.030	9.16± 0.150	8.58± 0.056
7	R1	9.38	8.74	8.12
	R2	9.32	8.84	8.24
	Mean ± SD	9.36± 0.0285	8.79± 0.067	8.19± 0.086
10	R1	8.73	8.15	7.54
	R2	8.59	8.33	7.68
	Mean ± SD	8.66± 0.094	8.24± 0.125	7.62± 0.096
15	R1	8.25	7.75	6.76
	R2	8.17	7.63	6.82
	Mean ± SD	8.21± 0.055	7.70± 0.084	6.80± 0.043
20	R1	7.30	6.98	6.22
	R2	7.29	7.02	6.13
	Mean ± SD	7.30± 0.003	7.01± 0.027	6.18± 0.065
30	R1	6.54	6.23	5.17
	R2	6.55	6.13	5.13
	Mean ± SD	6.55± 0.007	6.19± 0.067	5.15± 0.026
45	R1	5.79	5.17	3.79
	R2	5.58	5.28	3.95
	Mean ± SD	5.69± 0.147	5.23± 0.078	3.87± 0.106
60	R1	4.78	4.06	2.66
	R2	4.79	4.13	2.58
	Mean ± SD	4.78± 0.005	4.10± 0.053	2.62± 0.054
90	R1	3.28	2.93	2.40
	R2	3.27	2.58	2.34
	Mean ± SD	3.27± 0.005	2.76± 0.248	2.36± 0.027
120	R1	2.21	2.12	1.45
	R2	2.96	2.15	1.51
	Mean ± SD	2.75±0.651	2.14±0.007	1.48±0.016

Table 3: Persistence of Flupyradifurone in Entisol (CoochBehar soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Replicates	Residues (µg g^{-1})		
		Entisol (CoochBehar soil)		
		Air Dry	Field capacity	Submerged
0	R1	9.98	10.05	10.07
	R2	9.91	10.07	10.01
	Mean ± SD	9.94± 0.049	10.04± 0.053	10.04 ± 0.049
3	R1	9.64	9.94	9.64
	R2	9.63	9.96	9.63
	Mean ± SD	9.64± 0.008	9.95± 0.117	9.64± 0.008
5	R1	9.55	9.71	9.55
	R2	9.58	9.77	9.57
	Mean ± SD	9.56± 0.019	9.74± 0.117	9.56± 0.018
7	R1	9.48	9.47	9.48
	R2	9.42	9.46	9.42
	Mean ± SD	9.46± 0.105	9.45± 0.139	9.46± 0.105
10	R1	9.39	9.32	8.89
	R2	9.36	9.33	8.86
	Mean ± SD	9.37± 0.018	9.33± 0.137	8.87± 0.017
15	R1	9.28	9.15	8.37
	R2	9.30	9.19	8.29
	Mean ± SD	9.29± 0.055	9.17± 0.981	8.34± 0.054
20	R1	9.10	8.32	7.76
	R2	9.03	8.32	7.63
	Mean ± SD	9.07± 0.048	8.32± 0.145	7.67± 0.047
30	R1	8.74	8.17	7.53
	R2	8.74	8.14	7.53
	Mean ± SD	8.74± 0.000	8.15± 0.163	7.53± 0.000
45	R1	8.59	8.16	6.79
	R2	8.91	8.09	6.71
	Mean ± SD	8.75± 0.230	8.13± 0.047	6.75± 0.23
60	R1	8.46	7.69	6.46
	R2	8.75	7.77	6.45
	Mean ± SD	8.61± 0.205	7.70± 0.053	6.41± 0.205
90	R1	7.48	7.31	5.48
	R2	7.49	7.13	5.48
	Mean ± SD	7.48± 0.001	7.22± 0.133	5.48± 0.001
120	R1	6.40	6.04	4.58
	R2	6.44	6.01	4.52
	Mean ± SD	6.42±0.002	6.02±0.045	4.54±0.020

Table 4: Persistence of Fluopyram in Entisol (CoochBehar soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Replicates	Residues (µg g^{-1})		
		Entisol (CoochBehar soil)		
		Air Dry	Field capacity	Submerged
0	R1	9.70	10.02	9.77
	R2	9.73	9.82	10.07
	Mean ± SD	9.72± 0.023	9.92± 0.142	9.92 ± 0.22
3	R1	9.02	9.312	9.37
	R2	9.14	9.54	9.16
	Mean ± SD	9.08± 0.085	9.43± 0.164	9.27± 0.15
5	R1	8.86	9.03	8.73
	R2	8.70	9.06	8.87
	Mean ± SD	8.78± 0.109	9.05± 0.02	8.81± 0.098
7	R1	8.58	8.36	8.05
	R2	8.67	8.25	8.20
	Mean ± SD	8.63± 0.06	8.31± 0.08	8.13± 0.106
10	R1	8.15	7.95	7.50
	R2	8.21	7.80	7.64
	Mean ± SD	8.19± 0.038	7.88± 0.104	7.57± 0.094
15	R1	7.36	7.04	6.72
	R2	7.52	7.14	6.69
	Mean ± SD	7.44± 0.112	7.10± 0.072	6.71± 0.021
20	R1	6.87	6.31	5.66
	R2	7.06	6.29	5.73
	Mean ± SD	6.97± 0.128	6.31± 0.019	5.70± 0.049
30	R1	5.96	5.53	4.46
	R2	5.98	5.56	4.63
	Mean ± SD	5.97± 0.015	5.55± 0.021	4.55± 0.117
45	R1	5.13	4.27	3.23
	R2	5.23	4.39	3.10
	Mean ± SD	5.19± 0.067	4.33± 0.084	3.17± 0.09
60	R1	4.30	3.58	2.31
	R2	4.13	3.43	2.13
	Mean ± SD	4.22± 0.118	3.51± 0.100	2.23± 0.126
90	R1	3.19	2.56	1.15
	R2	2.99	2.28	1.03
	Mean ± SD	3.10± 0.143	2.42± 0.200	1.09± 0.083
120	R1	2.56	2.18	1.01
	R2	2.54	2.15	1.02
	Mean ± SD	2.52±0.263	2.16±0.054	1.01±0.236

Table 5: Persistence of Flupyradifurone in Alfisol (Ludhiana soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Replicates	Residues (µg g^{-1})		
		Alfisol (Ludhiana soil)		
		Air Dry	Field capacity	Submerged
0	R1	9.98	10.02	10.17
	R2	9.93	10.07	10.04
	Mean ± SD	9.96± 0.036	10.05± 0.031	10.11 ± 0.094
3	R1	9.94	9.74	9.61
	R2	9.95	9.75	9.69
	Mean ± SD	9.95± 0.003	9.75± 0.135	9.66± 0.055
5	R1	9.84	9.69	9.62
	R2	9.85	9.63	9.66
	Mean ± SD	9.83± 0.01	9.61± 0.025	9.69±0.03
7	R1	9.77	9.59	9.31
	R2	9.77	9.56	9.35
	Mean ± SD	9.77± 0.001	9.58± 0.022	9.33± 0.037
10	R1	9.52	9.36	9.85
	R2	9.57	9.42	9.85
	Mean ± SD	9.55± 0.17	9.40± 0.042	9.85± 0.037
15	R1	9.30	9.25	8.60
	R2	9.40	9.31	8.62
	Mean ± SD	9.36± 0.068	9.28± 0.23	8.61± 0.056
20	R1	9.14	9.11	8.21
	R2	9.19	9.18	8.42
	Mean ± SD	9.15± 0.040	9.15± 0.047	8.32± 0.15
30	R1	8.89	8.56	8.13
	R2	8.84	8.78	8.34
	Mean ± SD	8.87± 0.034	8.67± 0.16	8.24± 0.15
45	R1	8.27	8.58	7.55
	R2	8.25	8.58	7.54
	Mean ± SD	8.26± 0.010	8.58± 0.000	7.55± 0.046
60	R1	7.63	7.88	7.17
	R2	7.84	7.81	7.18
	Mean ± SD	7.74± 0.149	7.85± 0.049	7.18± 0.006
90	R1	7.18	6.76	6.08
	R2	7.04	6.78	6.16
	Mean ± SD	7.11± 0.096	6.77± 0.01	6.12± 0.058
120	R1	6.54	6.02	5.95
	R2	6.52	6.05	5.91
	Mean ± SD	6.53±0.056	6.04±0.004	5.93±0.120

Table 6: Persistence of Fluopyram in Alfisol (Ludhiana soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Replicates	Residues (µg g^{-1})		
		Alfisol (Ludhiana soil)		
		Air Dry	Field capacity	Submerged
0	R1	9.79	9.96	9.84
	R2	9.81	9.83	9.98
	Mean ± SD	9.80± 0.014	9.88± 0.074	9.96 ± 0.105
3	R1	9.32	9.49	9.61
	R2	9.41	9.65	9.50
	Mean ± SD	9.38± 0.047	9.57± 0.077	9.56± 0.067
5	R1	9.21	9.30	9.23
	R2	9.11	9.32	9.31
	Mean ± SD	9.15± 0.059	9.32± 0.011	9.28±0.047
7	R1	9.02	8.86	8.82
	R2	9.09	8.79	8.91
	Mean ± SD	9.06± 0.039	8.82± 0.048	8.86± 0.047
10	R1	8.73	8.59	8.49
	R2	8.77	8.49	8.57
	Mean ± SD	8.75± 0.18	8.53± 0.056	8.54± 0.046
15	R1	8.18	7.99	8.02
	R2	8.29	8.06	8.00
	Mean ± SD	8.26± 0.262	8.03± 0.033	8.01± 0.10
20	R1	7.85	7.84	7.79
	R2	7.98	7.82	7.63
	Mean ± SD	7.90± 0.070	7.83± 0.009	7.74± 0.087
30	R1	7.22	7.49	7.28
	R2	7.24	7.34	7.38
	Mean ± SD	7.23± 0.008	7.39± 0.090	7.35± 0.064
45	R1	6.66	6.66	6.74
	R2	6.73	6.57	6.66
	Mean ± SD	6.70± 0.036	6.61± 0.044	6.72± 0.053
60	R1	6.09	6.03	5.99
	R2	5.97	6.10	5.88
	Mean ± SD	6.01± 0.068	6.09± 0.060	5.95± 0.060
90	R1	5.33	5.02	4.69
	R2	5.19	4.84	4.62
	Mean ± SD	5.24± 0.080	4.94± 0.099	4.65± 0.044
120	R1	4.53	4.25	3.78
	R2	4.43	4.16	3.80
	Mean ± SD	4.47±0.048	4.18±0.061	3.79±0.022

Table 7: Persistence of Flupyradifurone in FYM under field capacity condition at 10 µg g^{-1} fortification level:

Days	Replicates	Field capacity
0	R1	9.68
	R2	9.68
	Mean ± SD	9.69± 0.004
3	R1	9.10
	R2	9.02
	Mean ± SD	9.06± 0.057
5	R1	8.38
	R2	8.38
	Mean ± SD	8.39± 0.0030
7	R1	7.62
	R2	7.47
	Mean ± SD	7.55± 0.0104
10	R1	6.67
	R2	6.70
	Mean ± SD	6.69± 0.021
15	R1	6.17
	R2	6.18
	Mean ± SD	6.18± 0.005
20	R1	5.74
	R2	5.69
	Mean ± SD	5.72± 0.0374
30	R1	4.62
	R2	4.63
	Mean ± SD	4.63± 0.004
45	R1	3.86
	R2	3.97
	Mean ± SD	3.92± 0.074
60	R1	3.12
	R2	3.09
	Mean ± SD	3.11± 0.020
90	R1	2.54
	R2	2.55
	Mean ± SD	2.55± 0.047
120	R1	1.14
	R2	1.15
	Mean ± SD	1.15±0.045

Table 8: Persistence of Fluopyram in FYM under field capacity condition at 10 µg g^{-1} fortification level:

Days	Replicates	Residues (µg g^{-1})
		FYM
		Field capacity
0	R1	10.05
	R2	9.93
	Mean ± SD	10.00± 0.086
3	R1	9.63
	R2	9.59
	Mean ± SD	9.61± 0.029
5	R1	9.05
	R2	8.97
	Mean ± SD	9.02± 0.052
7	R1	8.44
	R2	8.43
	Mean ± SD	8.44± 0.002
10	R1	7.82
	R2	7.87
	Mean ± SD	7.85± 0.033
15	R1	6.93
	R2	6.84
	Mean ± SD	6.89± 0.057
20	R1	6.03
	R2	5.99
	Mean ± SD	6.02±0.023
30	R1	4.70
	R2	4.79
	Mean ± SD	4.75± 0.067
45	R1	3.54
	R2	3.58
	Mean ± SD	3.56± 0.026
60	R1	2.59
	R2	2.62
	Mean ± SD	2.61± 0.022
90	R1	1.53
	R2	1.44
	Mean ± SD	1.49± 0.059
120	R1	0.92
	R2	0.94
	Mean ± SD	0.93±0.051

Table 9: Dissipation of Flupyradifurone in Inceptisol (IARI soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Dissipation %		
	Inceptisol (IARI soil)		
	Air Dry	Field Capacity	Submerged
0	-	-	-
3	6.79	-3.05	9.17
5	8.88	6.14	14.52
7	11.47	12.38	19.95
10	13.49	17.37	26.44
15	17.99	28.09	34.82
20	21.00	33.11	43.03
30	28.90	42.49	49.93
45	43.12	54.60	59.23
60	55.28	61.99	68.98
90	71.44	74.88	84.27
120	74.36	78.21	89.32

Table 10: Dissipation of Fluopyram in Inceptisol (IARI soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Dissipation %		
	Inceptisol (IARI soil)		
	Air Dry	Field Capacity	Submerged
0	-	-	-
3	2.24	2.59	6.08
5	4.01	6.28	13.55
7	7.74	9.99	17.48
10	10.26	15.64	23.22
15	14.93	21.19	31.48
20	24.36	28.27	37.69
30	32.15	36.69	48.06
45	41.06	46.49	60.94
60	50.44	58.06	73.54
90	66.07	71.76	89.63
120	68.15	75.29	93.56

Table 11: Dissipation of Flupyradifurone in Entisol (CoochBehar soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Dissipation %		
	Entisol (CoochBehar soil)		
	Air Dry	Field Capacity	Submerged
0	-	-	-
3	5.09	2.91	12.89
5	8.18	6.96	18.71
7	11.83	9.25	23.84
10	16.68	12.69	30.71
15	20.34	18.92	36.12
20	25.54	26.07	42.88
30	31.79	33.91	52.25
45	45.05	47.47	62.20
60	51.75	61.88	73.70
90	69.63	77.24	85.04
120	80.86	84.56	88.47

Table 12: Dissipation of Fluopyram in Entisol (CoochBehar soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Dissipation %		
	Entisol (CoochBehar soil)		
	Air Dry	Field Capacity	Submerged
0	-	-	-
3	5.40	-2.82	7.02
5	8.49	1.36	11.66
7	10.12	9.39	18.43
10	14.74	14.09	24.03
15	22.51	22.59	32.72
20	27.40	31.24	42.78
30	37.78	39.46	54.34
45	45.96	52.75	68.19
60	56.01	61.72	77.68
90	67.74	73.57	89.03
120	75.98	81.65	92.57

Table 13 : Dissipation of Flupyradifurone in Alfisol (Ludhiana soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Dissipation %		
	Alfisol (Ludhiana soil)		
	Air Dry	Field Capacity	Submerged
0	-	-	-
3	6.78	5.27	9.16
5	9.90	3.89	12.81
7	14.87	11.85	13.88
10	23.93	19.31	21.22
15	27.55	29.29	29.17
20	33.13	38.41	36.63
30	40.90	49.03	47.44
45	49.33	60.94	64.39
60	61.01	68.97	78.16
90	77.97	80.64	88.72
120	81.56	86.58	92.86

Table 14: Dissipation of Fluopyram in Alfisol (Ludhiana soil) under air dry, field capacity and submerged condition at 10 µg g^{-1} fortification level:

Days	Dissipation %		
	Alfisol (Ludhiana soil)		
	Air Dry	Field Capacity	Submerged
0	-	-	-
3	0.92	5.91	13.65
5	5.44	12.91	19.86
7	11.06	21.63	26.56
10	19.45	30.53	31.06
15	23.92	35.83	42.15
20	30.06	40.58	50.66
30	36.52	51.89	61.89
45	45.40	59.32	71.36
60	56.21	67.69	79.52
90	68.08	83.94	90.47
120	74.84	88.54	93.02

Table 21: Flupyradifurone leaching under varying amount water in Inceptisol (IARI soil): (BQL < 0.05 µg g^{-1})

Column depth (cm)	Residues in Soil (µg g^{-1})				Water added (ml)	Residues in leachate (µg mL^{-1})			
	R1	*R2*	*Mean*	% recovered		*R1*	*R2*	*Mean*	% recovered
0-5	15.75	14.04	14.89	74.47	**40**	0.07	0.08	0.07	0.36
5-10	1.20	1.28	1.24	6.2	**80**	0.16	0.18	0.17	0.80
10-15	0.31	0.28	0.29	1.46	**160**	0.32	0.30	0.31	1.60
15-20	BQL	BQL	BQL	BQL	**240**	0.58	0.59	0.58	2.91
20-25	BQL	BQL	BQL	BQL					

Table 22: Fluopyram leaching under varying amount water in Inceptisol (IARI soil): (BQL < 0.05 µg g^{-1})

Column depth (cm)	Residues in Soil (µg g^{-1})				Water added (ml)	Residues in leachate (µg mL^{-1})			
	R1	*R2*	*Mean*	% recovered		*R1*	*R2*	*Mean*	% recovered
0-5	13.74	12.71	13.23	66.12	**40**	0.05	0.05	0.05	0.25
5-10	0.85	1.06	0.95	4.77	**80**	0.10	0.09	0.09	0.53
10-15	0.19	0.21	0.20	0.98	**160**	0.25	0.27	0.26	1.28
15-20	BQL	BQL	BQL	BQL	**240**	0.47	0.45	0.46	2.36
20-25	BQL	BQL	BQL	BQL					

Table 23: Flupyradifurone leaching under varying amount water in Alfisol (Ludhiana soil): (BQL < 0.05 µg g^{-1})

Column depth (cm)	Residues in Soil (µg g^{-1})				Water added (ml)	Residues in leachate (µg mL^{-1})			
	R1	*R2*	*Mean*	% recovered		*R1*	*R2*	*Mean*	% recovered
0-5	11.74	12.07	11.9	59.53	**40**	BQL	BQL	BQL	BQL
5-10	0.94	0.89	0.92	4.58	**80**	BQL	BQL	BQL	BQL
10-15	BQL	BQL	BQL	BQL	**160**	0.06	0.05	0.05	0.31
15-20	BQL	BQL	BQL	BQL	**240**	0.12	0.10	0.11	0.62
20-25	BQL	BQL	BQL	BQL					

Table 24: Fluopyram leaching under varying amount water in Alfisol (Ludhiana soil): (BQL < 0.05 µg g^{-1})

Column depth (cm)	Residues in Soil (µg g^{-1})				Water added (ml)	Residues in leachate (µg mL^{-1})			
	R1	*R2*	*Mean*	% recovered		*R1*	*R2*	*Mean*	% recovered
0-5	12.42	10.99	11.7	58.55	**40**	BQL	BQL	BQL	BQL
5-10	0.98	0.95	0.96	4.82	**80**	BQL	BQL	BQL	BQL
10-15	BQL	BQL	BQL	BQL	**160**	BQL	BQL	BQL	BQL
15-20	BQL	BQL	BQL	BQL	**240**	0.52	0.51	0.51	0.26
20-25	BQL	BQL	BQL	BQL					

Table 25: Flupyradifurone leaching under varying amount water in Entisol (CoochBehar soil): (BQL < 0.05 µg g^{-1})

Column depth (cm)	Residues in Soil (µg g^{-1})				Water added (ml)	Residues in leachate (µg mL^{-1})			
	R1	*R2*	*Mean*	% recovered		*R1*	*R2*	*Mean*	% recovered
0-5	8.97	8.44	8.71	43.53	**40**	BQL	BQL	BQL	BQL
5-10	BQL	BQL	BQL	BQL	**80**	BQL	BQL	BQL	BQL
10-15	BQL	BQL	BQL	BQL	**160**	BQL	BQL	BQL	BQL
15-20	BQL	BQL	BQL	BQL	**240**	0.07	0.06	0.06	0.28
20-25	BQL	BQL	BQL	BQL					

Table 26: Fluopyram leaching under varying amount water in Entisol (CoochBehar soil): (BQL < 0.05 µg g^{-1})

Column depth (cm)	Residues in Soil (µg g^{-1})				Water added (ml)	Residues in leachate (µg mL^{-1})			
	R1	*R2*	*Mean*	% recovered		*R1*	*R2*	*Mean*	% recovered
0-5	7.89	8.29	8.09	40.45	**40**	BQL	BQL	BQL	BQL
5-10	BQL	BQL	BQL	BQL	**80**	BQL	BQL	BQL	BQL
10-15	BQL	BQL	BQL	BQL	**160**	BQL	BQL	BQL	BQL
15-20	BQL	BQL	BQL	BQL	**240**	0.06	0.05	0.05	0.26
20-25	BQL	BQL	BQL	BQL					

Table 27: Flupyradifurone leaching under continuous flow condition in Inceptisol (IARI soil):

Column depth (cm)	Residues in Soil ($\mu g\ g^{-1}$)			
	R1	***R2***	***Mean***	**% recovered**
0-5	15.47	15.52	15.49	77.47
5-10	1.33	1.11	1.22	6.09
10-15	0.53	0.89	0.71	3.56
15-20	BQL	BQL	BQL	BQL
20-25	BQL	BQL	BQL	BQL
Residues in leachate ($\mu g\ mL^{-1}$)				
400mL	0.97	1.18	1.08	5.38

Table 28: Fluopyram leaching under continuous flow condition in Inceptisol (IARI soil):

Column depth (cm)	Residues in Soil ($\mu g\ g^{-1}$)			
	R1	***R2***	***Mean***	**% recovered**
0-5	14.48	14.59	14.53	72.68
5-10	1.28	1.31	1.29	6.47
10-15	0.46	0.41	0.43	2.17
15-20	BQL	BQL	BQL	BQL
20-25	BQL	BQL	BQL	BQL
Residues in leachate ($\mu g\ mL^{-1}$)				
400mL	0.95	0.93	0.94	4.72

Table 29: Flupyradifurone leaching under continuous flow condition in Alfisol (Ludhiana soil): (BQL < 0.05 µg g^{-1})

Column depth (cm)	Residues in Soil (µg g^{-1})			
	R1	***R2***	***Mean***	**% recovered**
0-5	14.63	14.37	14.5	72.50
5-10	1.18	1.10	1.14	5.69
10-15	BQL	BQL	BQL	BQL
15-20	BQL	BQL	BQL	BQL
20-25	BQL	BQL	BQL	BQL
Residues in leachate (µg mL^{-1})				
400mL	0.24	0.21	0.22	1.15

Table 30:Fluopyram leaching under continuous flow condition in Alfisol (Ludhiana soil): (BQL < 0.05 µg g^{-1})

Column depth (cm)	Residues in Soil (µg g^{-1})			
	R1	***R2***	***Mean***	**% recovered**
0-5	13.69	13.42	13.56	67.78
5-10	0.98	1.04	1.01	5.07
10-15	BQL	BQL	BQL	BQL
15-20	BQL	BQL	BQL	BQL
20-25	BQL	BQL	BQL	BQL
Residues in leachate (µg mL^{-1})				
400mL	0.10	0.10	0.10	0.52

Table 31: Flupyradifurone leaching under continuous flow condition in Entisol (CoochBehar soil):

(BQL < 0.05 µg g^{-1})

Column depth (cm)	Residues in Soil (µg g^{-1})			
	R1	***R2***	***Mean***	**% recovered**
0-5	10.35	9.88	10.11	50.57
5-10	BQL	BQL	BQL	BQL
10-15	BQL	BQL	BQL	BQL
15-20	BQL	BQL	BQL	BQL
20-25	BQL	BQL	BQL	BQL
Residues in leachate (µg mL^{-1})				
400mL	0.09	0.08	0.09	0.45

Table 32: Fluopyram leaching under continuous flow condition in Entisol (CoochBehar soil):

(BQL < 0.05 µg g^{-1})

Column depth (cm)	Residues in Soil (µg g^{-1})			
	R1	***R2***	***Mean***	**% recovered**
0-5	9.99	9.20	9.59	47.99
5-10	BQL	BQL	BQL	BQL
10-15	BQL	BQL	BQL	BQL
15-20	BQL	BQL	BQL	BQL
20-25	BQL	BQL	BQL	BQL
Residues in leachate (µg mL^{-1})				
400mL	0.05	0.11	0.08	0.42