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PHYTOREMIDATION OF FIPRONIL AND MATALAXYL BY VIGNA RADIATA AND BRASSICA JUNCEA

Mohini R Natha
MSc Biochemistry, department of biochemistry, CBSH, S. D. A. U., Sardarkrushinagar

Dr. S. R. Vyas
Unit Head, Bioscience research centre, S. D. A. U., Sardarkrushinagar

Dr. S. B. Gondaliya
Associate research Scientist (Biochemistry), Bioscience research centre, S. D. A. U., Sardarkrushinagar

ABSTRACT

The Department of Biochemistry, College of Basic Science and Humanities, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, carried out the current study, "Phytoremidation of Fipronil and Matalaxyl by *Vigna radiata* and *Brassica juncea*."

Currently, weeds, pests, and diseases cause roughly 40.00 percent of the world's potential agricultural yield to be lost each year; however, this loss would easily treble without crop protection from pesticides. Growing food crops is not a simple undertaking. There are around 30,000 kinds of weeds, 3,000 species of worms, and 10,000 species of insects that feed on plants. From 1998 to 2005, the pesticide fipronil was frequently utilised in the US; this was followed by a sharp fall. In order to control superfluous arthropods in different types of food, horticulture, and turf plants, it was utilised in seed coatings and granular soil treatment. A fungicide called metalaxyl is often used on plant diseases such mildews, pythium, and late blight. Uridine inhibition in particular RNA polymerase inhibition are credited with the pesticide's efficiency. Metalaxyl is classified as having EPA toxicity class III. The stability of metalaxyl under hydrolysis and its resistance to destabilisation by light and water have both been proven by recent investigations. However, one of metalaxyl's characteristics is its capacity to permeate deep soil, which raises the possibility of groundwater contamination. Therefore, excessive usage of metalaxyl in the same location is to be avoided. Only the R-isomer of metalaxyl has been shown to be the chemically active substance. The current investigations were conducted to remediate by *Vigna radiata* and *Brassica juncea* with the aforementioned situation in mind. Fipronil and Metalaxyl were used in the experiment in five different combinations (T1: Untreated control, T2: Fipronil 5 percent SC @ 200 g a.i. ha-1, T3: Fipronil 5 percent SC @ 600 g a.i. ha-1, T4: Metalaxyl 35 percent WS @ 200 g a.i. ha-1, and T5: Metalaxyl 35 percent WS @ 600 g The research was divided into two sessions; the first session evaluated the persistence of Fipronil and Metalaxyl in sandy loam soil prior to the phytoremediation inquiry, and the second session assessed the effectiveness of *Vigna radiata* and *Brassica juncea* for phytoremediation.

The combined findings showed that *Vigna radiata* dramatically lowered the half-lives of Fipronil and Metalaxyl from 84 days to 45 days and from 67 days to 36 days, respectively. It's interesting to note that *Brassica juncea* remediates Fipronil and Metalaxyl more effectively thanks to their much-shortened half-lives of 3.7 days and 4.8 days, respectively. While the residues of Fipronil and Metalaxyl from the whole plant of *Brassica juncea* at harvest were below the determination limit, bioaccumulation of the chemicals was seen in the *Vigna radiata* at the 30th day after harvest. *Brassica juncea* is the perfect crop for phytoremediation because of this.
Keywords: phytoremediation, Fipronil, Matalaxyl, *Brassica juncea, Vigna radiata*

I.INTRODUCTION

By aerating the soil and neutralising the pollutants, plants use a process known as phytoremediation to change harmful bioaccumulated compounds into chemically stable ones. The remediation process (also known as rhizodegradation) causes the plant's volatiles (phytovolatilization) and organic harmful chemical to pass through the leaf tissues and, as a result, the microbial proliferation occurs (Suman *et al*., 2018). There are growing environmental and health issues due to the massive number of pesticides and heavy metals that have been released into the environment as a result of rapid modernization, industrialisation, and urbanisation (Ashraf *et al*., 2019).

The widespread use of pesticides endangers agricultural operations. However, phytoremediation may be a workable answer to this persistent issue (Liu *et al*., 2014). Pesticide removal from soil and developing crops is the main focus of phytoremediation. Reagents like the Fenton reagent that have been found are mostly for clean-up. The reagent is used to oxidise the insecticide, which is then transformed into by-products that are mostly safe (Baldissarelli *et al*., 2019). In an electron-Fenton reaction, Rodrigo *et al*. (2014) demonstrated that oxidising pesticides with Fenton reagent will result in hydrogen peroxide. Even it may be transported in a pesticide tank and administered with a sprayer.

Most pesticides are not biodegradable and cannot be broken down by microorganisms. As a result, they continue to affect people, living things in the soil, and the environment (Nurzhanova *et al*., 2013). Through phytoremediation, the plant may recover the toxicity of the pesticide-derived compounds. Rhizomediation, phytotransformation, and phytoaccumulation are only a few of the processes that go into phytoremediation (Liu *et al*., 2014). Some plants, including some transgenic plants, have effective phytoremediation abilities. Other plants may be utilised in phytoremediation procedures, such as the frequently used Jatropha curcas. Even now, research is being done to create transgenic plants that will greatly enhance the capacity of phytoremediation (Abioye *et al*., 2017). Pesticides that have contaminated bioaccumulated may harm people by altering the digestive system. To comprehend phytoremediation properly, it is necessary to have a deeper understanding of the intricate mechanisms and interactions between soil, plants, and microbes. Plants and the environment are likely to interact, which may lead to effective clean-up (Karthikeyan and Kulakow, 2003).

Pesticide-polluted agricultural lands are now a major problem worldwide. Even natural occurrences like soil erosion, storms, and floods have the potential to spread pesticides to uncultivated regions and pollute the untreated soil, providing a biological hazard to the inhabitants (both people and animals). A sample of soil contains a large number of pesticides, as shown by Silva *et al*. (2019). According to the study's findings, more than 50% of the analysed fields have used more than one pesticide, while 25% of the fields have only used one (Silva *et al*., 2019).

But these physiochemical methods have their own drawbacks, such as cost inefficiency, ineffectiveness in removing contamination when the concentration is low, irreversible changes to the physicochemical and biological properties of soil that worsen the soil ecosystem, and occasionally the production of some secondary pollutants (DalCorso *et al*., 2019). The idea of phytoremediation emerged as a result of the necessity to develop a professional, eco-friendly, and cost-effective method to recover pesticide-contaminated soil. It is a plant-based strategy that promotes the use of plants for elemental pollutant extraction and removal or for reducing their bioavailability in soil (Silva *et al*., 2019).

The removal of pesticides has been attempted several times. Recombinant microorganisms have been created utilising genetic engineering approaches to ensure optimal biodegradation of pesticides (Silva *et al*., 2019). Numerous studies have been conducted on genetic capacity, assessment, the creation of genetically competent microorganisms, and the development of efficient treatments in order to achieve this goal. In another instance, research on bioaugmentation, which may be utilised for phytoremediation and many other kinds of pesticides, has been investigated. The effectiveness of endophytes in remediation has also been mentioned. In addition to endophytes, using bacteria is another successful strategy (Cycon *et al*., 2017).

Even at extremely low quantities, ionic chemicals may be absorbed by plant roots via the soil. By establishing a rhizosphere ecosystem via their roots, plants may collect and change chemical pollutants in the soil, removing them from the environment and restoring the soil's fertility (Jacob *et al*., 2018). There are several benefits of the phytoremediation technique, some of which have been described here, according Jacob *et al*. (2018)

It protects the environment and preserves the ecosystem by minimising exposure to contaminants to both

- Installation and ongoing maintenance costs are relatively predictable and reasonable for an efficient autotrophic system that gets its energy from the sun.
- It can be used on a large-scale field, has a broad variety of applications, and is simple to dispose of afterwards.
- By stabilising pesticides, it prevents erosion and metal leaching, greatly reducing the chance of pollutants spreading laterally.
- Through the release of various organic materials into the soil, it improves soil fertility.

From 1998 to 2005, the pesticide fipronil was frequently utilised in the US; this was followed by a sharp fall. In order to control superfluous arthropods in different types of food, horticulture, and turf plants, it was utilised in seed coatings and granular soil treatment. Ticks, termites, and roaches may all be eliminated with the use of the gel-spray version of fipronil. Its other spray and spot solutions are widely used to keep pet animals free of fleas, ticks, and mites (James, 2013).

Both plants and animals get infected when intentionally or unintentionally exposed to a treated host, and because of its widespread environmental usage, it enters the food chain (Srivastava *et al*., 2011). It's not a volatile material, and although it clings to the soil and degrades there, this offers little protection against runoff and contaminating surface water. It has a greater propensity to bind in soils with a high concentration of organic carbon than in soils with a low concentration of organic carbon (Singh, *et al*., 2014). Its ability to attach to soil and biodegrade prevents it from seeping into the groundwater. Half-Life may range between 3 and 45 days (Fent, 2014).

Despite all of the aforementioned characteristics, Fipronil has been found in seas in the United States and other countries. Fipronil's environmental reactions include bio-reduction to generate Fipronil sulphide, which is then oxidised to form Fipronil sulfone (Singh *et al*., 2014). It responds differently on the soil's surface, where the sun's UV light causes the formation of de-sulfinyl fipronil (James, 2013). When considerable harmful amounts of the various equivalents and mixtures indicated above, including de-sulfinyl fipronil, are recorded from any area, they are also found there (Srivastava *et al*., 2011).

A fungicide called Metalaxyl, also known as N-(2, 6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester, is mostly used to treat plant diseases such mildews, pythium, and late blight. The unique qualities of metalaxyl include a significant safety profile and increased systemic action. In addition to inhibiting hyphal growth, metalaxyl also interferes with the development of sporangia and haustoria in oomycetes (Leadbeater, 2014).Uridine inhibition in particular RNA polymerase inhibition are credited with the pesticide's efficiency. Metalaxyl is classified as having EPA toxicity class III. The stability of metalaxyl under hydrolysis and its resistance to destabilisation by light and water have both been proven by recent investigations. However, it breaks down when exposed to UV light, and photosensitizers like TiO_2, H_2O_2, riboflavin, and humic acid may hasten this process. One of Metalaxyl's characteristics is that it may contaminate groundwater by penetrating deep soil. Therefore, excessive usage of metalaxyl in the same location is to be avoided. It has been noted that the only chemically active form of metalaxyl is its R-isomer (Sukul and Spiteller, 2000).

II.LITERATURE REVIEW

Fipronil

Fipronil is a broad-spectrum pesticide that is increasingly used to control domestic pests and to treat pets for flea and tick infestations. It is often found in Frontline and Max force products. Fipronil works as a GABA antagonist, which causes unnecessary CNS stimulation and insect death. GABA receptor-equipped insects do it more effectively than mammalian counterparts. Human exposure is mostly inadvertent and frequently causes headaches and dizziness. Upper respiratory tract discomfort, nausea, and vomiting are the main symptoms in kids. Seizures may happen with a severe consumption (Zhan *et al*., 2021). The course of therapy is mostly determined by the kind of exposure and the symptoms, with seizures being a common symptom in people with high exposure. As part of the therapy, benzodiazepines are used often to reduce drowsiness and manage seizures. The diagnosis shouldn't be made based just on pupils' ability to rule something in or out. Tachycardia should not prevent the administration of atropine, and the therapy should be based on signs and symptoms rather than acetylcholinesterase levels (James, 2013).

Fipronil exposure has been linked to reports of clinical traits related to cholinesterase inhibition. Asthma brought on by chemicals in organophosphate pesticides, systemic toxic effects of pyrethroids, etc., were among the symptoms (Gutta *et al*., 2019). A phenyl pyrazole insecticide, fipronil primarily affects the gastrointestinal tract and central nervous systems. A lot of people are reporting kidney injury. Fipronil usage has also been linked to incidences of liver damage. After receiving supportive therapy for three weeks, there was a clinical improvement (Gutta *et al*., 2019). In the US, it has been extensively utilised for crop protection, indoor pest management, and animal health in the best spray and spot-on forms that are now available. As a non-competitive channel blocker for GABA-gated chloride channels, it is supposed to work. administering a drug that contains fipronil Products have been proven to be less harmful when ingested orally, topically, orally. But the veterinary product might become hazardous if it is accidentally injected or licked. The study's findings showed that veterinary products may result in localised skin irritation or hair loss at the application site. Additionally, it was shown that dogs were more responsive to the drug Fipronil than rats. Fipronil usage in young or tiny rabbits was linked to lethargic behaviour, anorexia, convulsions, and mortality (Webster, 1999). Fipronil has a low to moderate level of tenacity, and because of photodegradation, hydrolysis, and volatility, it degrades in the environment. It quickly converts in plants into fipronil sulphone, which has a 327-day half-life and is dependent on the substrate and environment of the afflicted plant. Fipronil has a strong affinity for the soil, although it has moderate soil mobility and little potential to contaminate groundwater. The half-life is 2.4 hours in a pH 12 environment, however it is more stable in aerobic conditions than anaerobic or alkaline ones (Duhan *et al*., 2015).

Metalaxyl

Frequently used, very mobile, persistent, and leaching fungicide Metalaxyl (Teixeira *et al*., 2011). Wu *et al*. (2019) investigate the impact of low-level exposure to metalaxyl on the zebrafish embryo's developing and functioning heart. According to the research, metalaxyl is a pesticide that is often used to treat illnesses brought on by Peronosporales. By exposing them to three different doses of metalaxyl, the harmful effects were examined in zebrafish embryos—the intended target organism—where cardiac function was examined. The study's findings showed that exposure to metalaxyl increased the incidence of heart bleeding, cardiac dysfunction, and pericardial edoema. Additionally, it was shown that as compared to solvent control larvae, metalaxyl-exposed larvae subjected to 50 and 500 ng/litre had considerably larger stroke volume in cardiac output (Gong *et al*., 2020).

Phytoremediation

According to Zhou *et al*. (2020), soil pollution by heavy metals and pesticides is a serious environmental problem. When heavy metals and pesticides interact with the soil, the situation gets more complicated, necessitating the need to recover the soil from their snares. The study's findings showed that bioremediation is a beneficial technique, especially for wide regions with little pollution. It may be used to address the impacts of both heavy metals and pesticides at the same time since it is affordable, readily accessible, and does no damage to the environment. Abiotic and biotic parameters including pH, temperature, biological competitiveness, and the biological condition of the plant are all connected to the bioremediation of heavy metals and pesticides, according to research results. The usage of certain plants and microorganisms that aid in the bioremediation of polluted soil was covered in depth in this research.

Phytoremediation through *Brassica juncea*

The effectiveness of mustard (*Brassica campestris* Linn.) and maize (*Zea Maize*) in removing the organochlorine insecticide Endosulfan was examined by and Kumar in 2012. Endosulfan's disappearance rates were 0.03684, 0.23490, and 0.17272 per day for unplanted treatments that were subsequently planted with mustard and maize, respectively. This indicated that plant uptake and phytoextraction with maize and mustard contributed 47.2 and 34.5 percent, respectively, and other degradation processes took up 38.7 and 35.9 percent, respectively, to the removal of the applied Endosulfan from the soil. After growing the crops mustard and maize in the soil, the accumulated Endosulfan dropped by 55.00 to 91.00 percent, indicating that plant uptake and phytoextraction may be the primary processes for Endosulfan removal by the plant.

Rani and Juwarkar (2012) assessed the capacity for bacterial isolates to break down phorate in soil and investigated the influence of plant species, *Brassica juncea*, on the biodegradation process. Three isolates, Ralstonia eutropha, Pseudomonas aeruginosa, and Enterobacter cloacae, make up the bacterial consortium. These isolates were acquired by enrichment on phorate, and they can degrade up to 73 3 percent in aqueous medium and 55 4 percent in sandy loam soil. When B. *Juncea* was present, the consortium's ability to degrade phorate in the soil rose by up to 64 5%. Phorate in the soil biodegraded at a rate of 38 4 % in the absence of plants compared to 15 2 % in controls.

White *et al*. (2005) investigated the inorganic element accumulation in 10 plant species in p,pV-dichlorodiphenyldichloroethane (p,pV-DDE) polluted soil under field settings. The plant species included both monocots and dicots, as well as two important groups within the dicots: the Brassicaceae and the Fabaceae. The plant species included rye, mustard, canola, vetch, pigeon pea, clover, peanut, and three cultivars of white lupin. A negative phosphorus treatment entailed adding AlSO4 to the soil before planting; duplicate mounds of each species received periodic amendments with nitrogen (N), phosphorus (P), and nitrogen and phosphorus combined (N/P). The proportion of p,pV-DDE phytoextracted was more than doubled in Mustard, Canola, and Peanut and was closely connected with a two-fold increase in total plant biomass.

Mehta *et al*. (1997) examined the Metalaxyl adsorption and degradation in mustard (*Brassica juncea*) plants after treatment as a seed dressing, foliar spray, or a combination of both under subtropical conditions in India. After being used as a seed dresser, metalaxyl absorption rose for up to 30 days before starting to drop and disappearing after 60 days. The greatest metalaxyl residues were found one day after spraying. The dispersion of metalaxyl following first deposits on mustard plants was almost complete after 15 days of spraying.

Tebuconazole, cypermethrin, and fipronil disappear from mustard greens and green onions grown in Central Vietnam. A first-order kinetic model was used to explain the dissipation data for each insecticide. Fipronil diffused the fastest of the studied chemicals, according to a comparison of the dissipation rate constants obtained under the identical crop and growth conditions for each chemical. Additionally, studies have demonstrated that crops grown in protected plots outlast those grown in uncontrolled plots in terms of pesticide persistence. The half-lives of tebuconazole, cypermethrin, and fipronil ranged from 0.9 to 3.3 days, 2.0 to 6.0 days, and 0.4 to 2.2 days, respectively (Chau *et al*., 2020).

III. RESEARCH METHODOLOGY

Fipronil and Metalaxyl dissipation was studied in the first part of the present research, and *Vigna radiata* and *Brassica juncea* bio efficiency was investigated in the second part of the study using a pot experiment. The experiment focused mostly on *Vigna radiata* and *Brassica Juncea*'s phytoremediation of Fipronil and Metalaxyl. The whole of these portions of the current research is provided below.

Experimental site

The current experiment was conducted at Sardarkrushinagar Dantiwada Agricultural University's Bio-Science Research Center (Gujarat).

Physico-chemical properties of soil

Composite soil samples were taken from the Agronomy Instructional Farm, Department of Agronomy, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar to determine the physico-chemical properties of the experimental sites. The field sample was taken at a depth of 0–15 cm, and it was then analysed for the different physical and chemical properties of the soil. According to data on soil analysis, the soil at the experimental location had a loamy sand-like texture and reacted somewhat alkalinely.

Collection and preparation of soil samples

Before treatments, soil samples from the top 0 to 15 cm of the experimental locations were taken using a V-shaped auger. Each plot included four sample points, which were collected and combined to create a composite sample. The soil was then crushed and put through a 2 mm plastic screen before being air dried (20-25°C) with relative humidity between 20 and 60 percent, and lastly kept in fabric bags until further investigation.

Mechanical analysis

Utilizing the Hydrometer technique as proposed by Black (1965) and Piper, mechanical analysis was carried out (1966). The soil sample was put into a 250 mL beaker weighing 50 g. It was then given 150 mL of distilled water and 10 mL of a 5 percent sodium hexametaphosphate solution. The material was well mixed before being put into a 1000 mL cylinder to achieve the desired volume. After carefully inserting the hydrometer into the cylinder and setting the suspension on the table for 20 seconds to allow for thorough mixing, the first reading was taken after 40 seconds. Additionally, the temperature was observed at the same time. Following two hours, readings were recorded continuously. These data were used to compute the percentages of sand, silt, and clay.

Determination of soil pH

The soil-water suspension technique was used to determine the pH of the soil (Jackson, 1973). 40 mL of distilled water and 20 g of soil were swirled at least four times over the course of 30 minutes (time required for the soil and water to attain equilibrium). The soil-water solution was agitated one again after 30 minutes, and the pH of the soil was then determined using a pH metre.

Determination of electrical conductivity of soil (dS/m)

Using Systronic's conductivity metre, the electrical conductivity was measured and represented as dS/m (Jackson, 1973). To get a clear supernatant, 20 g of soil were intermittently agitated in 40 mL of distilled water 4-5 times before being left overnight. With KCl solution, the electrical conductivity bridge was calibrated. Freshly produced distilled water was made into 1L after 0.7456 g of dry KCl was dissolved in it. At 25°C, this solution had a 1.40 dS/m electrical conductivity. The solution's temperature (in degrees Celsius) and electrical conductivity (in decibels per metre) were measured using the clear supernatant of the soil-water suspension, which had been left overnight.

Bulk density (g/cm) of soil

An air-dry clod that served as a good example was picked, and a length of thread was gently tied around it, leaving approximately 20 cm free. Weighed and hung in the air was the clod. Holding the end of the free thread, momentarily submerge the clod in the melted paraffin, then let the extra drip off. The clod and adhering paraffin are weighed after the paraffin has solidified. Weighted the clod once more after suspending it in water with a paraffin covering.

Maximum water holding capacity of soil

Filter paper and a cap were used to weigh an empty metal cup. After taking off the top, dry dirt gently tapered into the metal cup. used a spatula to level the ground and taped the brass cup. A brush was used to clean the excess dirt before placing the brass cup's cap on top. The dirt and cap were then added to the Brass cup for weighing. A petri dish holding water was filled with the brass cup, which should have been completely immersed.

Determination of organic carbon (%)

Preparation of reagents

- N Potassium dichromate (K2Cr2O7) solution was made by combining 1 L of distilled water with 49.04 g of AR grade K2Cr2O7.
- 392 g of ferrous ammonium sulphate were dissolved in distilled water to create a 0.5 N ferrous ammonium sulphate solution. After that, 15 mL of concentrated AR-grade sulfuric acid was added, and 2 L of distilled water was added to the mixture.
- Powdered sodium fluoride (95 percent)
- Sulfuric acid in concentrated form 36 N (AR grade)
- Diphenylamine indicator was created by combining 0.5 g of the substance with 20 mL of distilled water and 100 mL of concentrated H2SO4 before being placed in the colour bottle.

Procedure

The quantity of oxidizable organic carbon in a soil sample was measured using the chromic acid titration technique (Walkely and Black, 1934). The process included combining 0.5 g of soil with 20 mL of concentrated H2SO4 solution and 10 mL of 1N K2Cr2O7. The solution was left to cool at ambient temperature. In 100 mL of distilled water, two grammes of NaF powder (a flocculating agent) were dissolved. The objects were violently shook. Ten drops of the indicator diphenylamine were added to the solution (The solution turned violet in color). An iron ammonium sulphate solution with a concentration of 0.5 N was then used to titrate the contents. Results for the blank and test samples were computed after measuring the volume of the ferrous ammonium sulphate (FAS) solution.

Procedure followed

A neutral ammonium acetate solution of 250 mL was poured to a 500 mL conical flask containing 10 g of soil, and the mixture was stirred periodically for an hour before being kept overnight.

Table: Physicochemical properties of the experimental site

Particular			Soil depth (0-15 cm)		Methods adopted
			For persistency study	For Bio-efficiency study	
[A]	Mechanical fractions:				
	1	Course sand (%)	44.88	44.48	International Pipette Method (Piper, 1966)
	2	Fine sand (%)	39.56	39.53	
	3	Silt (%)	7.08	7.09	
	4	Clay (%)	4.75	7.43	
	5	Texture class	Sandy loam		
[B]	Chemical properties:				
	1	Soil pH (1:2.5, soil: water ratio)	7.65	7.66	Potentiometric (Jackson, 1973)
	2	Electrical Conductivity (dsm^{-1} at 25ºC)	0.17	0.16	Conductivity metric (Jackson, 1973)
	3	Bulk density(g/cm^3)	1.3	1.3	Clod saturation method (Disturbed soil)
	4	Maximum Water Holding Capacity (%)	55	54	Measurement using Brass-cup with perforated base Wet-digestion
	5	Organic carbon (%)	0.34	0.34	Walkley and Black method (Walkley and Black, 1934)
	6	Cation Exchange Capacity(cmol (+)/kg)	8	8	Ammonium Acetate method at pH 7.0 (Jackson, 1973)

The materials had been filtered using Whatman No. 44. The filtrate was collected in a 100 mL measuring flask. Using 20 to 25 mL at a time, the neutral solution was used to repeatedly leach the soil onto the filter paper, letting the leachate fully drain before adding a fresh aliquot. The leachate was collected and saved for cation-specific analysis. The total cation exchange capacity of the soil will be determined using the residue that was left on the filter paper. The dirt that was still on the filter paper was continuously washed with 60 percent ethanol in order to get rid of the extra ammonium acetate. After moving the soil with the filter paper into a distillation flask, 200 mL of water and 2-3 gramme of MgO were added. After steam distillation, the distillate was collected in a known excess of 0.1 N sulfuric acid, to which a few drops of methyl red were added. With 0.1 N NaOH, the excess acid was titrated back.

Experimental details

The specifics of the investigation's experimental procedures are provided below.

1	Crop	:	***Vigna radiata*** **and** ***Brassica juncea***	
		:	*Vigna radiata*:	GM5
2	Variety	:	*Brassica juncea*:	GDM 4
3	Design	:	Completely Randomized Design (CRD)	
4	Replications	:	Six	

Sampling

One hour after applying pesticides, zero-day soil sample was done. In a container, the earth was gathered from two to three locations and gathered there.

Certified Reference Material (CRM)

Sigma Aldrich provided certified reference materials for the drugs fipronil (98.0 percent purity) and metalaxyl (99.2 percent purity). The usual preparation employed only high-grade organic solvents.

Primary standard

Using the "ACZET" balance, a technical grade (CRM) standard (10 mg) was precisely weighed (on a basis of 100 percent purity) (maximum capacity 220 g and sensitivity 0.01 mg). The standards were then transferred to a volumetric flask with a 100 mL capacity (A grade). Acetonitrile was used to dissolve the substance at first, and acetonitrile made up the whole volume in the end, giving each pesticide a concentration of 100 mg L-1.

Secondary/Intermediate standards

A 5 mL aliquot of the main standard was diluted to 25 mL in a volumetric flask with acetonitrile, yielding a concentration of 20 mg L-1.

Final working standards

Final working standards were created using the intermediate standard. Acetonitrile was used to dilute the appropriate aliquots to the necessary final volume, resulting in a final concentration of 0.01, 0.025, 0.075, and 0.1 mg L-1.

Method validation studies

Different matrices, including entire plants and sandy loam soil, were used to validate the approach in terms of recovery studies, linearity, accuracy, precision, LOD, and LOQ. The recovery research was conducted at levels of 10 X LOQ and 20 X LOQ.

Linearity study

To assess instrument performance, a linearity study was conducted. The detector response (height/area) v/s concentration graph was plotted to determine the linearity. Seven distinct working standard concentrations—0.01, 0.025, 0.075, and 0.1 mg L-1—were injected, and their responses (V) were recorded to determine the linearity. For GCMS and LCMSMS, the standard injection volumes were respectively 1.0 L and 5.0 L. The best-fit linear connection model was used to get a correlation coefficient and equation.

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

The LOD and LOQ were determined prior to quantifying the pesticide matrices, such as soil and the whole plant. To achieve signal-to-noise ratios of 3:1 for LOD and 1:10 for LOQ, matrix-match standard injection was used in liquid chromatography.

Recovery study

Before beginning the analysis of the test sample for each treatment, the accuracy (percent recovery) and precision (percent RSD) were calculated to guarantee quality assurance information. For the recovery investigation, 100 g of soil in triplicate samples were taken from untreated plots. In order to assess residues, a representative 10 g of sample was fortified with a combination of Fipronil and Metalaxyl herbicides at 0.05 and 0.1 ppm levels. The fortified samples were then left at room temperature for two hours.

Residues and dissipation study of Fipronil and Metalaxyl in soil

Solvents and reagents

Water, tri-sodium citrate dihydrate, sodium hydrogen citrate, sodium chloride (NaCl), magnesium sulphate (MgSO4), primary secondary amine (PSA), acetonitrile, and sodium chloride (NaCl).

Apparatus

Weighing balance, Vortex mixer, Refrigerated centrifuge.

Instruments

Gas Chromatography (GCMS: Thermo Scientific MS-ITQ 700)

Liquid Chromatography-Mass Spectrometry (LC-MS/MS: UHPLC, API 8050-Shimadzu).

Extraction procedure

A 50 mL centrifuge tube was filled with a 10 g typical soil sample. The tube was then left at room temperature for 15 minutes while 3 mL of cold water and 10 mL of acetonitrile were added for extraction. 3 g of magnesium sulphate, 0.75 g of sodium chloride, 0.750 g of trisodium citrate, and 0.370 g of disodium hydrogen citrate were added to this, and the mixture was violently agitated for five minutes. The sample was centrifuged for 10 minutes at a speed of 6000 rpm. A sample of 8.0 mL was taken, transferred to a 15 mL centrifuge tube, and then incubated for 1 hour at -10°C in a deep freezer before being centrifuged for 5.0 minutes at 4500 rpm. A sample with a volume of 6.0 mL was taken, transferred to a centrifuge tube with a capacity of 15 mL, and then vortexed for 2.0 minutes with 0.6 g of MgSO4 and 50 mg of PSA. The extract was then centrifuged for 10 minutes at a speed of 6000 rpm. 4 mL of the extract were dried in a TurboVap® device with nitrogen at 45 °C. The final volume was then reduced from this to 1 mL using acetonitrile, filtered through a 0.22 m syringe filter, and then examined for the presence of fipronil and metalaxyl using GCMS and LC-MS/MS (Anastassiades *et al*., 2003).

- 10 g soil sample transferred in to 50 mL centrifuge tube

- Acetonitrile and mixture of salts added.
- Vortexed to mix.
- Centrifuged and transferred supernatant in another 10 mL centrifuge tube.
- Vortexed to mix.
- Centrifuged sample.
- After evaporation and reconstitution, transferred supernatant in to auto sampler vial.

Residues of Fipronil and Metalaxyl in *Vigna radiata* and *Brassica juncea* whole plant after periodic harvest

Collection of *Vigna radiata* and *Brassica juncea* samples

For the purpose of residue analysis, two plants from each pot of *Vigna radiata* and *Brassica juncea* were taken and pooled with the relevant treatments.

Extraction of pesticide from the whole plant of *Vigna radiata* and *Brassica juncea*

Solvents and reagents

Acetonitrile, water, acetic acid, sodium acetate, magnesium sulfate (MgSO4), primary secondary amine (PSA).

Apparatus

Weighing balance, mortar and pestle, vortex mixer, refrigerated centrifuge.

Instruments

Liquid Chromatography-Mass Spectrometry (LC-MS/MS: UHPLC, API 8050-Shimadzu).

LC-MS/MS analysis of Fipronil and Metalaxyl

For the LC-MS/MS analysis, a Shimadzu Prominence series HPLC system (Shimadzu, Tokyo, Japan) and LC-MS/MS system-8050 were utilised. The LC-20 AD binary pump, SIL-20 AC autosampler, DGU-20A5 online degasser, and CTO-20A column oven were all included in the HPLC system. The ESI source and the collision cell both employed nitrogen that was produced from a Peak Scientific nitrogen generator (Billerica, MA, U.S.A.) with a purity of at least 99 percent. Agilent Fortis C18 column measuring 2.1 100 mm 2.6 m with a 10 L injection was used for the analysis. The column's temperature stayed at 35°C all the time. A mobile phase gradient with a flow rate of 0.3 mL min1 and a composition of water with 2 mM Ammonium formate and 0.05 percent formic acid, and methanol with 2 mM Ammonium formate and 0.05 percent formic acid, was utilised. Eluent A, which is HPLC-grade water with 2 mM ammonium formate and 0.05 percent formic acid, and Eluent B, which is HPLC-grade methanol with 2 mM ammonium formate and 0.05 percent formic acid, made up the mobile phase (eluent B). This is how the gradient elution was carried out: 0.01-0.2 minutes: 5% B; 0.2-7.5 minutes: 50% B; 7.5-11 minutes: 90% B; 11-14.60 minutes: 98 percent B; 14.60-17 minutes: 5% B.

An ESI source was used to run the mass spectrometer in both positive and negative modes. Ion spray voltages of 5.5 kV (ESI+) and 4.5 kV (ESI), source temperature of 600 °C, interface temperature of 350 °C, curtain gas of 35 psi (nitrogen), ion source gas of 55 psi, ion source gas of 50 psi, and collision gas of 5 psi (nitrogen) were the ESI parameters. In both positive and negative polarities, ESI-MS/MS was carried out in planned multiple reaction monitoring mode (MRM) by scanning four precursor/product ion transitions for the two target analytes.

GSMS analysis of Fipronil and Metalaxyl

For the separation and identification of pesticides, a gas chromatography (Thermo Scientific MS-ITQ 700) outfitted with a split/splitless injector system was employed. Helium was used as the carrier gas in the GCMS at a constant flow rate of 1.2 mL/min. Thermo Scientific's TG-5MS (30 m 0.25 mm 0.25 m) capillary column was used for separation. A split flow of 50 L/min was used to inject a volume of 1 L of extract. At 280 C, the injection port was maintained. The oven was set to an initial temperature of 90°C for 3 minutes, then raised to 180°C at a ramp rate of 25°C/min, increased to 280°C at a ramp rate of 5°C/min, and held at 290°C for 5 minutes.

IV. ANALYSIS

"Bio-efficacy and persistence of Fipronil and Metalaxyl in sandy loam soil under *Vigna radiata* and *Brassica juncea* growth" was the focus of the inquiry.

In vitro research was undertaken at the Bio-science Research Center, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, to ascertain the dissipation of Fipronil and Metalaxyl in soil (Gujarat). Fipronil (5 percent SC) and Metalaxyl (35 percent WS) were administered at 200 and 600 g a.i. ha-1 to the corresponding pot. The soil samples were taken and the residues analysed at 0 (1 hour), 1, 3, 5, 10, 15, 20, 25, 30, 35-, 40-, 45-, and 50-days following application. To investigate the fipronil and metalaxyl dissipation in accordance with the steps outlined in the chapter's materials and methodology. The pertinent meteorological information, such as the temperature, was noted in the materials and procedures.

In this chapter, the findings of the inquiry are presented together with statistical conclusions. The following sub-headings are used to display and explain the findings.

- Method validation studies for Fipronil and Metalaxyl from different matrices
- Dissipation of Fipronil and Metalaxyl in sandy loam soil
- Dissipation of Fipronil and Metalaxyl under *Vigna radiata* cultivated soil
- Periodical analysis of Fipronil and Metalaxyl residues from *Vigna radiata* plant
- Dissipation of Fipronil and Metalaxyl under *Brassica juncea* cultivated soil
- Periodical analysis of Fipronil and Metalaxyl residues from *Brassica juncea* plant
- Phytotoxicity of Fipronil and Metalaxyl on studied crops

Method validation studies for Fipronil and Metalaxyl from different matrices

The process of ensuring that the analytical technique employed for a particular test is suitable for its intended application is known as method validation. Results of method validation may be used to evaluate the calibre, dependability, and consistency of analytical findings; it is a crucial element of any efficient analytical procedure (Huber, 2007). In order to evaluate the presence of Fipronil and Metalaxyl in unidentified samples, a number of validation metrics, including linearity, method detection limits, or LOD and LOQ, accuracy (mean percent recovery), and precision, were established (percent RSD). A linearity analysis was done to assess the effectiveness of the Mass Spectrometry (MS) detector. For the linearity investigation, a graph of detector response v/s Fipronil and Metalaxyl concentration was created, and the correlation equation and co-efficient were obtained. The accuracy of the methods employed to analyse Fipronil and Metalaxyl from different matrices was evaluated using recovery studies. The accuracy of the approach is shown by the mean recovery from such studies. Percent Relative Standard Deviation was used to measure the analytical method's accuracy (percent RSD). The LOD and LOQ were determined prior to quantifying Fipronil and Metalaxyl in plant matrix and soil samples.

Linearity study

A linearity study was done to assess the MS detector's performance. A graph of detector response v/s concentration was created for the linearity investigation (Fig. 4.1 and Fig. 4.2). Equivalent amounts of Fipronil and Metalaxyl were injected into the MS Detector to verify their linearity, and the resulting responses were recorded at 0.005, 0.01, 0.025, 0.05, and 0.1 ppm. Fipronil and Metalaxyl were found to be linear in the range of 0.05 to 0.1 ppm, according to the findings from the linearity study (Figs. 4.1 and 4.2). For Fipronil and Metalaxyl, the R2 values that were computed from the correlation equation using a positive linear correlation model (y = a + bx) were 0.9998 and 0.9988, respectively.

Fipronil and Metalaxyl's responses to different dosages on the MS Detector are shown in Figs. 4.1 and 4.2. Linearity was assessed using these data.

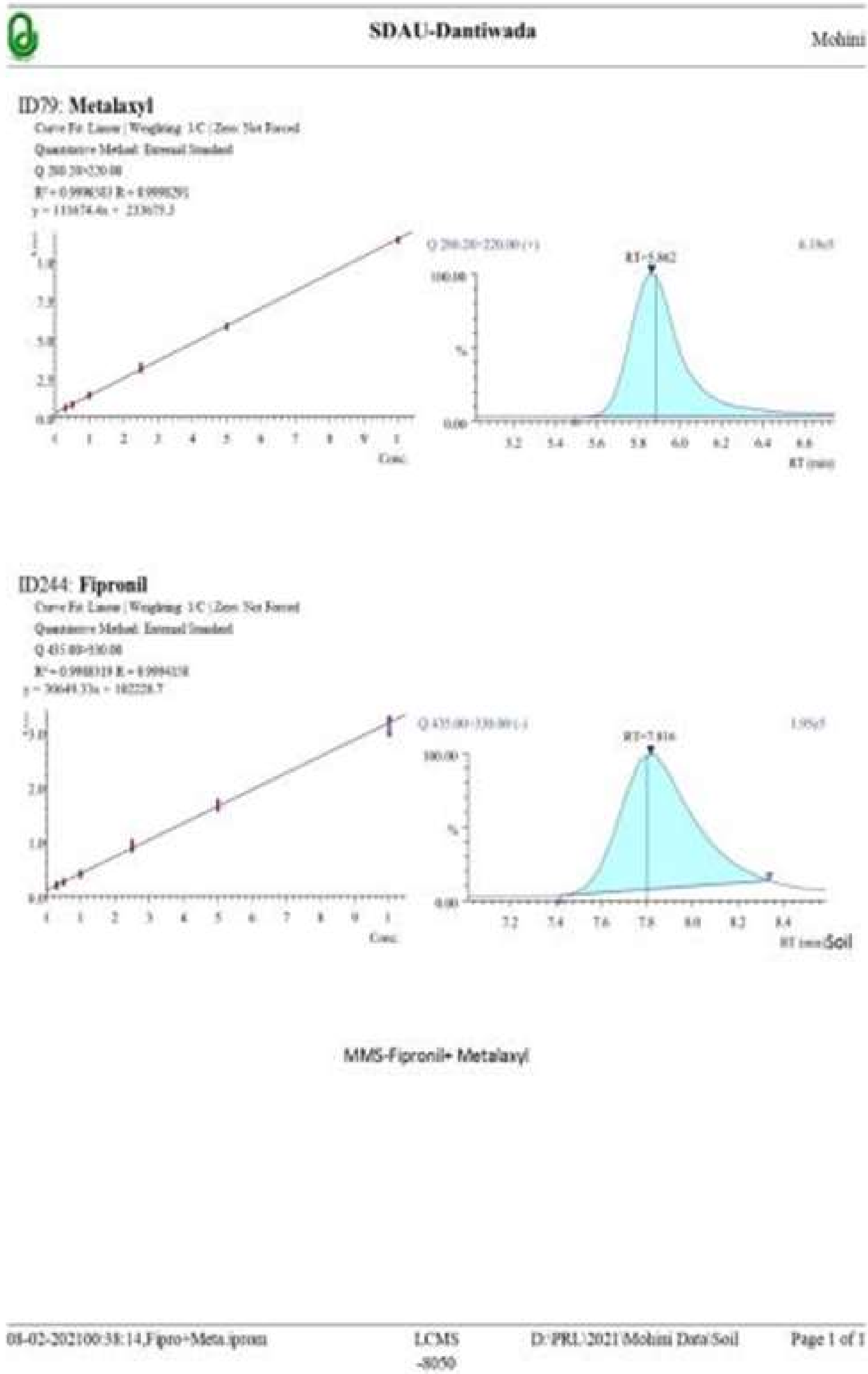

Fig. 4.1: Response of Fipronil and Metalaxyl (Soil MMS) on MS at different concentrations

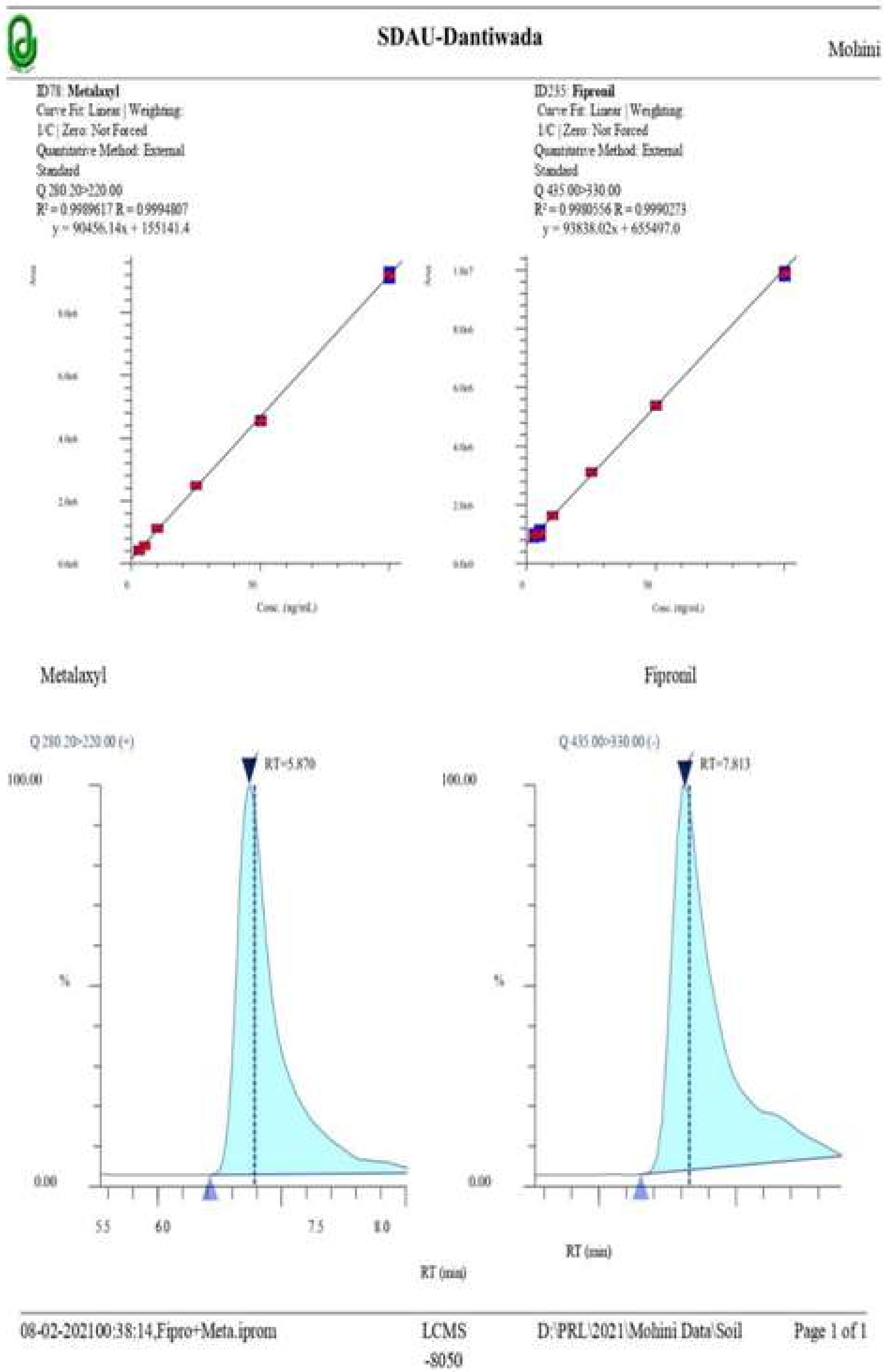

Fig. 4.2: Response of Fipronil and Metalaxyl (Vigna radiata and Brassica

LOD and LOQ

The final sample volume for the plant and soil was 5 g/mL. The injected samples for soil and plant were 5.0 L and 1.0 L, respectively. After taking into account all of these factors, it was found that the LOQ (S/N ratio > 10) was 0.005 ppm (0.005 g/g) and the LOD (Method Detection Limit-MDL) was 0.002 ppm (0.002 g/g), which is 1/3 of the LOQ for LCMS MS. While the LOD (Method Detection Limit-MDL) for GCMS MS was found to be 0.02 ppm (0.02 g/g) and the LOQ to be 0.05 ppm (0.05 g/g), respectively.

Statistics showed that LCMS MS had 10 times bigger LOD and LOQ than GCMS, hence it was decided to use LC-MS/MS for future investigation.

Validation studies (accuracy and precision)

Validation of Fipronil and Metalaxyl extraction method for sandy loam Soil

Fipronil and Metalaxyl were administered for a recovery study in sandy loam soils prior to the persistence research. Following two separate spike levels of 0.05 and 0.1 g g-1, soil samples were examined using the procedures outlined in section 3.8 of the materials and methods chapter. Three replications, a control, a reagent blank, and three replications altogether made up the recovery experiment.

The recovery study's findings showed that, at spiking levels of 0.05 and 0.1 ppm, respectively, the mean recovery of fipronil from soil was 100.87 8.73 percent and 103.23 6.02 percent. RSD percentages of 8.65 and 5.83 were noted at the comparable spike level. At spiking doses of 0.05 and 0.1 ppm, respectively, the mean recovery of Metalaxyl from soil was 97.03 4.97 percent and 107.33 5.24 percent. The respective spike levels' percent RSD were 5.12 percent and 4.88 percent. Fipronil and Metalaxyl were extracted using the analytical method, which was found to be accurate and precise because the mean recoveries were in the range of 91.52-102.2 percent and 96.62-108.66 percent, respectively, and the corresponding RSD ranged from 2.35- 9.12 and 3.34- 19.41 percent. These values were within the limits set by the SANTE (2017) guidelines, which are below 20 percent. Any analytical technique that reports mean recovery in the range of 70–120 percent and percent RSD < 20 percent is accurate and precise, according to the SANTE (2017) standards.

V.CONCLUSION

Fipronil and Metalaxyl were used in the experiment in five different combinations (T1: Untreated control, T2: Fipronil 5 percent SC @ 200 g a.i. ha-1, T3: Fipronil 5 percent SC @ 600 g a.i. ha-1, T4: Metalaxyl 35 percent WS @ 200 g a.i. ha-1, and T5: Metalaxyl 35 percent WS @ 600 g Prior to the examination into phytoremediation, the first session of the study was carried out to gauge the persistence of Fipronil and Metalaxyl in sandy loam soil.

The effectiveness of *Vigna radiata* and *Brassica juncea* for phytoremediation was assessed in the second segment. The soil was treated with the identical amounts of Fipronil and Metalaxyl in pot soil, and 10 seeds were sown in each pot in a sextuplet pattern after 0 (1 hour) of soil sample collection. Samples of the soil were taken after 0 days, 5, 10, 15, 20, 25, and 30 days. However, *Brassica juncea* and *Vigna radiata* entire plant samples were taken at intervals of 10, 20, and 30 days.

In the years 2020–2021, the Pesticide Residue Laboratory examined the levels of Fipronil and Metalaxyl residues in soil and the whole plant from pot experiments of *Vigna radiata* and *Brassica juncea* at harvest.

In this chapter, the findings of the inquiry are presented together with statistical conclusions. The following sub-headings are used to display and explain the findings.

Method Validation Studies for Fipronil and Metalaxyl from Different Matrices

In order to evaluate the effectiveness of the MS detector, a linearity study was conducted. An equal amount of five different doses of the combination of Fipronil and Metalaxyl, namely, 0.01, 0.025, 0.05, 0.075, and 0.1 and 1.0 mg L-1, were injected, and their corresponding responses were recorded, in order to prove the linearity of Fipronil and Metalaxyl on MS Detector. According to the findings from the linearity investigation, the concentration range of 0.01 to 0.1 mg L-1 was determined to be linear for both Fipronil and Metalaxyl. For Fipronil and Metalaxyl, the R2 values that were computed from the correlation equation using a positive linear correlation model (y = a + bx) were 0.9998 and 0.9988, respectively.

The analytical technique used to extract Fipronil and Metalaxyl from the whole *Vigna radiata* plant was determined to be accurate and exact since the mean recovery fell between 91.94 and 110.01 and 92.87 and 104.05 percent, respectively. While the analytical method used to extract Fipronil and Metalaxyl from soil was found to be accurate and precise as mean recoveries were in the range of 91.52-102.2 percent and 96.62-108.66 percent respectively, the mean recovery of Fipronil and Metalaxyl from the entire plant of *Brassica juncea* was found to be accurate and precise in the range of 88.04-111.11 percent and 90.30-101.34 percent.

Dissipation of Fipronil and Metalaxyl in Sandy Loam Soil

In order to determine the level of Fipronil and Metalaxyl residue in sandy loam soil, soil samples were taken from the pot on days 0, 1, 3, 5, 7, 10, 15, 20, 25, 30, 35, and 50 (one hour after treatment). With the treatment of Fipronil @ 200 g a.i. ha-1 (T2) and 600 g a.i. ha-1 (T3), the initial deposits of Fipronil were determined to be 80.53 and 258.70 g kg-1 at zero day and correspondingly. On the third day, the comparable Fipronil residue levels were 80.14 and 254 g kg-1. The residue level continued to decrease, reaching 72.73 and 244.87 g kg-1 on 10 days, passing at close to BDL level on 50 days at first treatment, while alternative therapy had 178.13 g kg-1 Fipronil residue level. For the first treatment, regression equations were obtained as follows: y = -0.0038x + 3.9052 (R2 = 0.9815), and for the second treatment, y = -0.0035x + 4.4216 (R2 = 0.944). The average half-life (t1/2) of fipronil was calculated to be 84 days based on the regression equation.

Initial deposits for the metalaxyl applications at 200 g a.i. ha-1 (T4) and 600 g a.i. ha-1 (T5) were 79.82 g kg-1 and 258.18 g kg-1, respectively. which decreased over the course of 72 hours to 78.30 g kg-1 and 252.43 g kg-1. After that, the residual loss was slow and reached 74.05 g Kg-1 and 238.36 g Kg-1, respectively, 10 days after application. The residue level was BDL for the treatment at 200 g a.i. ha-1 (T4) and 155.08 g Kg-1 for the treatment at 600 g a.i. ha-1 at 45 days after the subsequent application (T5). With a half-life (t1/2) of 68.40 days and an R2 value of 0.9895, the regression equation for the dissipation of Metalaxyl application at 200 g a.i. ha-1 was derived as y = -0.0044x + 3.9109.

Dissipation of Fipronil and Metalaxyl Under Vigna radiata Cultivated Soil

With treatment Fipronil @ 200 g a.i. ha-1 (T2) and 600 g a.i. ha-1 (T3), the first deposits of the drug were discovered to be 82.69 and 260.87 g kg-1 at zero days, respectively. On the tenth day, the equivalent Fipronil residue concentrations were 72.09 and 227.66 g kg-1. On the 30th day, the residual levels of fipronil with the corresponding treatments were 52.57 g kg-1 and 161.95 g kg-1, respectively.

With treatments of Metalaxyl at 200 g a.i. ha-1 (T4) and 600 g a.i. ha-1 (T5), respectively, at zero days, the first deposits of the chemical were calculated to be 83.18 and 264.66 g kg-1. The corresponding Metalaxyl residual levels on day five were 71.72 and 272.21 g kg-1. On the 30th day, the Metalaxyl residue levels for the therapy Metalaxyl @ 200 g a.i. ha- and 141.76 g kg-1 for the alternative one reached BDL.

Periodical Analysis of Fipronil and Metalaxyl Residues from Vigna radiata Plant

Fipronil and Metalaxyl initially deposited in *Vigna radiata* at 10 days were BDL for both higher and lower authorised dosages of the corresponding pesticides. With respect to time, the agglomeration of fipronil and metalaxyl gradually rises. Accumulation during the 30th day of harvesting established the maximum for each pesticide, including both treatments.

Dissipation of Fipronil and Metalaxyl under Brassica juncea Cultivated Soil

With treatments with Fipronil at 200 g a.i. ha-1 (T2) and 600 g a.i. ha-1 (T3), respectively, at zero days, the first deposits of the drug were observed to be 79.62 and 260.42 g kg-1. The amounts of residue were measured after 0, 5, 10, 20, and 30 days and decreased with time. Fipronil had identical residual levels of 56.26 and 187.93 g kg-1 on day 5. On the 10th day for treatment with 200 g of fipronil a.i. ha- and the 20th day for therapy with 600 g a.i. ha-1, the fipronil residue levels declined further and reached BDL.

With treatments Metalaxyl @ 200 g a.i. ha-1 (T4) and @ 600 g a.i. ha-1 (T5), the Metalaxyl residue in sandy loam soil grown with *Brassica juncea* on 0 days was 82.35 and 259.75 g g-1, respectively. In response to this, Metalaxyl level declined and approached BDL level after 10 days after treatment Metalaxyl at 200 g a.i. ha-1 (T4) and 15 days later at 600 g a.i. ha-1 (T5).

Periodical Analysis of Fipronil and Metalaxyl Residues from Brassica juncea Plant

At 10 days, the first deposits of Fipronil and Metalaxyl were 55.60 g kg-1 for Fipronil @ 200 g a.i. ha-1 (T2) and 59.70 g kg-1 for Metalaxyl @ 200 g a.i. ha-1 (T4), respectively, and 166.70 g kg-1 and 151.04 g kg-1 for Fipronil @ 600 g a.i. ha-1 (T3) and Metalaxyl @ 600 g a.i. ha-1 (T5),. The residual amounts of Fipronil and Metalaxyl had been eliminated by the 20th day of analysis, which included all treatments, and had reached BDL.

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