“Biotic and Abiotic Degradation of Imidacloprid in Soil and Water”

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By
Kamlesh Kumar Vishwakarma

Research Supervisor
Dr Prakash B Samnani

DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
VADODARA-390002

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INTRODUCTION

Pesticides constitute one of the most hazardous groups of contaminants to human health, fauna and environment in general. Majority of such substances are applied directly to soil or sprayed over crop fields and hence released directly to the environment. Therefore, pesticides can contaminate surface waters directly as spray drift or run-off and also via drainage through the soils of treated farmland. The amount and type of pesticides in the water of a particular area depends largely on the intensity of production and type of crops being cultivated. Farmers have increased the use of pesticides to increase crop yield per acre. Pesticide residues enter aquatic environments through effluent release, discharges of domestic sewage and industrial wastewater, atmospheric deposition, runoff from agricultural fields, leaching, equipment washing, and disposal of empty containers and direct dumping of wastes into the water systems (Yang et al., 2005). The distribution of various pesticides contaminants in the marine and estuarine environment depends on the physical and chemical properties of the ecosystem as well as the partition coefficients of individual chlorinated hydrocarbons (Sarkar et al., 1997). Pesticide residues remaining in soil constitutes hazards of it being incorporated in next crop cycle. Due to these issues, there is greater concern about their determination in various environment segments.

This work exclusively deals with determination of Imidacloprid pesticide in soil and in water. Imidacloprid is a neonicotinoid insecticide belonging to chloronicotinyl nitro guanidine chemical family. Chemically, Imidacloprid, [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-lideneamine], is a common pesticide used in India. Imidacloprid was firstly introduced by Bayer Agricultural Products (Daraghmeh *et al.,* 2006). Imidacloprid, though marketed as an insecticide which has a high activity against sucking insects, also acts as an early plant growth enhancer.

The Summary is divided into Five Chapters:

Chapter 1

Literature Survey

Chapter 2

Analytical method development and validation of imidacloprid for trace Determination of imidacloprid residue in/on soil and water by HPLC.

(a) Method Optimization
(b) Method Validation
(c) Soil Characterization

Chapter 3: Biotic and Abiotic degradation of imidacloprid in/on different types of soil

(a) Residue of imidacloprid in soil in biotic and abiotic conditions

(b) Half life calculation

(c) Degradation of imidacloprid in different soils

(d) Effect of soil characteristic on persistence of imidacloprid

(e) Effect of dose concentration on persistence of imidacloprid

Chapter 4

Biotic and abiotic degradation of imidacloprid in water at different *pH* 4, 7, 9

(a) Residue of imidacloprid in water at ***p*H 4, 7, 9**

(b) Half life calculation

Chapter 5: **Determination of imidacloprid in vegetables (Cabbage and Spinach) and soil by Ultra Fast Liquid Chromatography (UFLC) with UV Detection**

(a) Extraction of imidacloprid from vegetable samples using solid phase extraction

(b) Extraction of imidacloprid from vegetable samples using QuEChERS Method

(c) Method validation

Chapter 1

Literature Survey

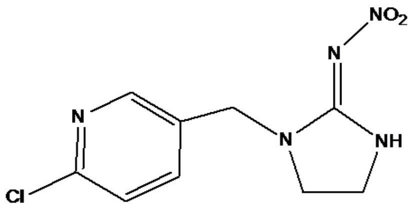
The use of synthetic pesticides as crop protection chemicals cannot be fully substituted in modern agriculture. Enhancement of crop production can be achieved only by the application of new pesticides. The effects of pesticide usage must be seen also in the context of soil pollution and sustainability of the agro-ecosystem. Increasing pesticide usage in agriculture adds to the rise in concern for the environmental contamination (Zhu *et al*., 2003). The presence of pesticide residues in ground water is extremely hazardous to human beings as ground water is major source of drinking water. Imidacloprid is used as insecticide with soil, seed and foliar applications for the control of sucking insects including rice hoppers, aphids, trips, whiteflies, termites, turf insects, soil insects and some beetles. It is most commonly used on rice, cereal, maize, potatoes, vegetables, sugar beets, fruit, cotton, hops and turf, and is especially systemic when used as a seed or soil treatment. Kidd *et al.* 1991 reported that the chemical works by interfering with neuronal transmission of insects. Different pesticides pose varying degrees and types of risk to water quality. It is reported that approximately three million people are poisoned and 200,000 die each year around the world from pesticide poisoning, the majority of them from the developing countries (FAO, 2000). Anindita et al. 2013 reported that the application of pesticide should be restricted to recommended doses only for sustenance of soil health. Additional research is suggested as result from laboratory studies may differ from field experiment.

Objectives:

In view of the forgoing account, the present investigation was designed to understand the persistence of imidacloprid in different types of soil and water at *p*H 4, 7 and 9 in biotic and abiotic conditions. The following methodology was followed to study the persistence of imidacloprid in soil and water.

- Development of analytical method for determination of trace level imidacloprid residue in/on soil and water by HPLC.
- Method validation
- Biotic and Abiotic degradation of imidacloprid in/on different soils.
- Impact of concentration of imidacloprid on persistence
- Impact of soil characteristic in persistence of imidacloprid
- Degradation of imidacloprid in water at different *p*H
- Recent techniques SPE and Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method applied for trace level determination of imidacloprid in vegetables.

Structure of Imidacloprid



[1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-lideneamine]

Chapter-2

Development and validation analytical method for determination of trace level imidacloprid residue in/on soil and water by HPLC

A number of methods have been reported to measure residues of imidacloprid, e.g., near-infrared spectroscopy (Pigeon, 2000), photo chemical fluorimetric method (Vilchez *et al.* 1996), electrochemical method, enzyme-linked immune sorbent assays (Wantatable, *et al.* 2004) capillary electrophoresis, gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography HPLC (Zhou Q *et al.* 2003). Among these methods GC and LC are the most suitable methods. Poor volatility and polarity poses limitations on the analysis by GC. Imidacloprid can be analyzed by GC after derivatization. The derivatization process does not lead to good recovery and reproducibility. We developed a rapid and sensitive HPLC method with high recovery rates to quantify imidacloprid in soils and water. The present study will concern extensively to trace level determination of imidacloprid in soil and water. A rapid, sensitive and reliable HPLC method was developed and validated for the determination of imidacloprid residue in water at different *p*H and in soil. Quantification was performed by reversed phase HPLC system equipped with UV detector. For determination of imdacloprid residues in/on different soil viz. sandy loam, clay, red and black soil and waters viz. *p*H 4, 7 and 9 using HPLC; a simple clean-up procedure using column chromatography was employed to yield sufficiently clean samples.

Method Optimization:

Analytical method to determine imidacloprid content by using HPLC was developed by selecting suitable instrument parameters and solvent to get clear resolution and separation of compound of interest.

Method Validation:

The method for the estimation of imidacloprid was validated. The validation parameters optimized were

a) Specificity
b) Linearity (LDR)
c) Limit of Detection (LOD)
d) Limit of Quantification (LOQ)
e) Precision (%RSD)
f) Accuracy (%Recovery)

Specificity

The standard sample and the control sample solutions in acetonitrile were injected into a High Performance Liquid Chromatography (HPLC) using suitable mobile phase and separation parameters were optimized. The interference (if any), in the determination of active ingredient in test substance was reported. Interference should not be more than 3% to the total peak area measured for target active content.

Linear Dynamic Range (LDR)

Five different concentrations of imidacloprid reference standard were prepared and injected into HPLC in duplicate. The linear calibration curve was established by plotting the mean peak area against concentration (mg/L). The correlation coefficient (r), intercept (a) and slope (b) were estimated.

Limit of Detection (LOD)

The minimum quantity of imidacloprid, which could be detected by the HPLC with signal to noise ratio of 3 ± 0.5, was calculated as limit of detection (LOD).

Limit of Quantification (LOQ)

The minimum quantity of imidacloprid, which could be quantified by the HPLC with signal to noise ratio between 5 and 10, was calculated as limit of quantification (LOQ).

Precision (%, RSD)

Determination of Repeatability

Five replicate injections of single sample solution of imidacloprid along with sequential injections of standard solution were injected into HPLC using the selected parameters. The active content in each sample injection, mean of active ingredient content and % RSD were calculated and reported.

Calculation of Precision (% RSD)

Precision was defined by the relative standard deviation (%, RSD). The precision (% RSD) was calculated as followed.

$$\text{Precision (\%, RSD)} = \frac{Standard\ deviation}{Arithematic\ mean\ (a.i.content)} \times 100$$

Accuracy (% Recovery)

Accuracy (% recovery) of the analytical method was determined in fortified soil and water samples and extracted samples. The samples was fortify at LOQ and 10 X LOQ levels for soil and water *P*H 4.0, 7.0, and 9.0.

Calculation of % Recovery (Accuracy)

% Recovery for active ingredient will be calculated as follows:

The linear calibration curve was established by plotting mean peak area of standard of imidacloprid solutions against concentrations (mg/L). The regression constant viz. slope (b), intercept (a) and correlation co-efficient (r) were calculated. The (a.i. content) of imidacloprid was calculated using the following formula:

$$Y = bX + a$$

$$X = \frac{Y - a}{b} \times D$$

Where,

Y = Peak area of the sample
a = Intercept
b = Slope
D = Dilution factor

Calculation of % Recovery (Accuracy

$$\% \text{ Recovery (Accuracy)} = \frac{\text{Concentration Recovered}}{\text{Concentration Fortified}} \times 100$$

The limit of quantitation (LOQ) was found to be 0.02 mg/kg in soil and 0.02 mg/L in water. The limit of detection (LOD) was 0.006 mg/kg in soil and 0.006 mg/L in water. Recoveries for imidacloprid were 95.18, 94.66, 95.27 and 94.78 % in black, red, sandy loam and clay soils respectively. Recoveries for imidacloprid from water were 96.86, 86.14, and 92.34 % at *p*H values of 4.0, 7.0, and 9.0 respectively. The detector linearity and the repeatability of the method proved to be very good.

Soil Characterization

The soil characteristics were studied to determine the physico-chemical properties of soil viz., *p*H, organic carbon, Water Holding Capacity (WHC), sand content (particle size distribution), and moisture content. The soil samples were collected from different places of Gujarat.

a) Valvada
b) Bardoli
c) Umarsadi
d) Baroda

Procedure for Soil Collection

The test soil was collected from a pasture land at a depth of 20 to 25 cm. The field was free from any chemical and biological contamination. A quantity of 5 kg well aerated moist soil was collected from different spots of the land at pre monsoon period. The soil was transferred to the laboratory on the collection day, after manual removal of stones and raw plant materials. The soil was sieved through 2.0 mm sieve and the moisture content was maintained between 40 to 60% of the maximum water holding capacity, using distilled water. The soil was stored at 4.0 ± 2.0 °C in refrigerator.

Chapter 3

Biotic and abiotic degradation of imidacloprid in/on different types of soil

Biotic factors are living or once-living organisms in the ecosystem. These are obtained from the biosphere and are capable of reproduction. Examples of biotic factors are animals, birds, plants, fungi, and other similar organisms. A detailed study of the available literature revealed that the majority of the pesticide degradation studies were carried out in normal agricultural soils.

Abiotic factors refer to non-living physical and chemical elements in the ecosystem. Abiotic resources are usually obtained from the lithosphere, atmosphere, and hydrosphere. Examples of abiotic factors are water, air, soil, sunlight, and minerals.

Role of biotic and abiotic factors other than photo-degradation in attenuating imidacloprid residues in soil were also studied.

Effect of soil characteristic on persistence of imidacloprid in soil

A detailed study of the available literature revealed that the majority of pesticide degradation studies were carried out in normal agricultural soils. However, there are a large part of arable soils, which are naturally or anthropogenically saline. Role of biotic and abiotic factors other than photo-degradation in attenuating imidacloprid residues in soil were reported. The degradation of pesticides on temperate soils cannot be extrapolated to tropical soils. Das *et al.*, 2007 reported the effect of novaluron on microbial biomass, respiration and hydrolyzing activity in tropical soils.

In the present work, the research efforts were directed to study degradation behavior of imidacloprid in different type of Gujarat soils under laboratory conditions. The soil characteristics play major role in the degradation of pesticides.

Persistence of imidacloprid was studied under laboratory conditions in biotic and abiotic conditions on four types of soil viz. sandy loam, clay, red and black soils following treatment at 1.0, 2.0 and 4.0µg/g fortification levels. Imidacloprid degradation has been found to be more rapid in soil with cover crop than continual availability for uptake by roots (Mullins, et al. 1992b). Thus imidacloprid can persist in soil depending on soil type, *p*H, use of organic fertilizers and preseence or absence of ground water. So a study was planned to determine imidacloprid residue in soil in biotic and abiotic condition under the laboratory control conditions. The imidacloprid residue in the soil samples were measured by validated analytical method (chapter -2). The pesticides concentration and soil characteristics such as *p*H, temperature, moisture and cation exchange capacity are very important for degradation of imidacloprid in soil under laboratory conditions. Pesticides reaching the soil may affect non-target soil microorganisms, thereby disturbing pesticide degradation processes (Pal *et al.*, 2006a). The overall dissipation of a pesticide from soil results from an interaction of biotic and abiotic mechanisms (Racke, *et al.*, 1997). The pesticide degradation in soils depends largely on their physico-chemical properties and how they interact with the biotic and abiotic soil components.

The dissipation of imidacloprid was found to be faster in black soil followed by sandy loam, clay and red soil with half life value of 50.10, 42.74 and 45.69, respectively. The half life value varied depending on concentration of imidacloprid in soil. The faster dissipation in black soil could be attributed to higher *p*H (7.47) and high organic matter (0.86%) as compared to other soil. The degradation of imidacloprid in soil depends upon organic carbon content, moisture and soil *p*H. In general the imidacloprid were found safe from environmental contamination point of view as their half-life values were less than 50 days in all other soils.

Chapter 4

Abiotic degradation of imidacloprid in water at different *p*H 4.0, 7.0 and 9

This study was conducted to determine the rate of hydrolysis and half-life of imidacloprid in buffer solutions of *p*H 4.0, 7.0 and 9 at 50 ± 0.5 ºC. The study was conducted in compliance with OECD Principles of GLP (1998) following the guideline of European commission, for Classification, Packaging and Labeling of Dangerous Substances in The European Union, Part 2- Testing Method, C.7, "Degradation – Abiotic Degradation Hydrolysis as a Function of *p*H", (January 1997). The hydrolytic degradation of imidacloprid after 5 days of incubation at 50 ± 0.5 °C at *p*H 4.0, 7.0 and 9.0 was found to be less than 10%. Therefore, it is concluded that the theoretical half-life imidacloprid is >1 year at 25 ºC and imidacloprid is hydrolytically stable and unlikely to hydrolyze under the environmental conditions at *p*H 4.0, 7.0 and 9.0. The compound is hydrolytically stable under neutral *p*H.

Biotic degradation of imidacloprid in water at different *p*H 4.0, 7.0 and 9.0

This study was conducted to determine the biotic degradation of imidacloprid and half-life in buffer solutions of *p*H 4.0, 7.0 and 9.0 at 25 ± 2 ºC. The present investigation was, therefore undertaken to evaluate the degradation in biotic conditions. The buffer solutions at different *p*H fortified and kept in laboratory conditions and samples were taken for analysis 0, 1, 3, 7, 15, 30, 45, 60 and 90 days after application. The degradation of imidacloprid after 90 days 72.5% at *p*H 9 and half life 52 days in biotic condition. The lowest degradation rate of imidacloprid was 52.45% at *p*H 7 in biotic condition. Therefore, it is concluded that the theoretical half-life imidacloprid is varied from 47 to 60 days at *p*H 4 in different dose levels. The degradation of imidacloprid at *p*H 7 less than *p*H 4 and *p*H 9, it means imidacloprid stable in neutral water *p*H.

Chapter 5

Determination of imidacloprid in vegetables (Cabbage and Spinach) and soil by Ultra Fast Liquid Chromatography (UFLC) with UV Detection

Several methods have been reported for the analysis of imidacloprid. Although imidacloprid residues can be analyzed by derivatization and gas chromatography, high-performance liquid chromatography (HPLC) has been already used and appears to be a suitable alternative because of the thermo-lability and polarity of imidacloprid. The LC method gave good results for imidacloprid in groundwater, soils, and fruits and vegetables but the limit of detection (LOD) was still too high for the present purpose (Srivastava Ashutosh K et al. 2012). Furthermore, the required method must satisfy strict quality criteria to be classified as a trace levels determination and low levels extraction procedure. We developed a new extraction scheme with high recovery rates, coupled to a UFLC methodology in order to extract and quantify imidacloprid in soils, vegetables cabbage and spinach. The present study will concern extensively to trace level determination of imidacloprid in vegetables (cabbage and Spinach) and soil by validating and using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) and solid phase extraction method followed by Ultra Fast Liquid Chromatography (UFLC). Comparison between (QuEChERS) extraction method and solid phase extraction for its efficiency and sensitivity were carried out. More generally, such a method can be easily adapted for the analysis of fruits and vegetables

A simple clean-up procedure using PSA was found to yield sufficiently clean samples. A quick, simple extraction procedure for the determination of imidacloprid in vegetables (cabbage and Spinach) and soil samples with good percentage recovery (89.39-99.52%) in all cases was developed. The limit of detection was 0.005µg/g. A method was developed using solid-phase extraction (SPE) and QuEChERS Method with Ultra Fast Liquid Chromatography (UFLC). The method was validated using Cabbage, Spinach and soil samples spiked with imidacloprid at different concentration levels (LOQ 0.01, 10 × LOQ 0.10 and 50 × LOQ 0.50 µg/g). Average recoveries (using each concentration 5 replicates) ranged 89.39 -99.5%, with relative standard deviations less than 2.15%, calibration solutions concentration in the range 0.005 -1.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.005 µg/mL and 0.01 µg/mL respectively. The satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained and according to the SANCO guidelines. It has been proved that the proposed method and extraction procedure provides a good sensitivity; reproducibility and recovery. The results demonstrated that the proposed extraction technique is a viable alternative for determination of imidacloprid in vegetables, soil and fruit samples. The vegetable samples were analyzed by LC-MS Ion Trap for imidacloprid peak conformation and other matrix effect.

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Prakash Samnani, and K.Vishwakrma 2013. *Persistence study of Imidacloprid in different soils under laboratory conditions* in International Journal of Environmental Sciences. Volume 4, No 2, 2013

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