Introduction

Pesticides are inherently toxic molecules. The pesticide residues can reach water bodies by leaching and runoff. Once they reach the water bodies, they start adversely affecting the aquatic environment. Presence of pesticides residue in ground water is extremely hazardous to human beings as ground water is major source of drinking water. Tisler T *et.al*. (2009) have reported that imidacloprid was persistent in water samples and was not readily biodegradable in aquatic environment. The hydrolysis study of pesticides is important for to understand hydrolysis pathway and stability of pesticides and degradation product. The present investigation was, therefore, undertaken to evaluate the rate of hydrolysis and half-life of imidacloprid technical in buffer solutions of *p*H 4.0, 7.0 and 9.0, at 50 ± 0.5 ºC. The study was conducted following the guideline of : European commission, for Classification, Packaging and Labelling 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). Wei Zheng et.al 1999 has reported the hydrolysis of imidacloprid in water at acidic, neutral and basic conditions. Little hydrolysis of imidacloprid in acidic and neutral conditions was observed. Under basic conditions hydrolysis of imidacloprid is highly *p*H and temperature dependent, higher the temperature and *p*H increases the hydrolysis of imidacloprid. They have reported one hydrolysis product,1-[(6-chloro-3-pyridinyl) methyl]-2- imidazolidone.

This chapter is divided into two sections, reporting studies carried out in abiotic and biotic conditions, respectively.

Section I: Abiotic Degradation of Imidacloprid

Materials and Methods: as per chapter 2

Method Validation

The specificity of the analytical method was studied by injecting solvent, reference standard solution, and control water sample into HPLC. There was no interference of the components with each other. The linearity was established by injecting five different concentrations, viz. 0.02 µg mL^{-1} to 5.00 mL^{-1}, and determining the response of the compound; these were fitted by linear regression to assess the linearity. Detection Limit (signal-to-noise ratio = 3 ± 0.5:1) was established.

The precision (% RSD) of the analytical method was determined by five replications in duplicate injection of fortified substrate soil and water extracts at LOQ level. The accuracy (% recovery) of the method was determined by five replications in duplicate injection of fortified substrate soil and water extracts at LOQ and 10 times LOQ levels. Precision (% RSD) should not exceed 20 %. The details of method validation procedure is discussed in **chapter-2**

Preparation of Buffer Solutions

Buffer (*p*H 4)

The buffer solution (*p*H 4) was prepared by mixing 90.0 mL 0.1 N sodium hydroxide solution with 500 mL 0.1M monopotassium citrate solution in a 1000 mL volumetric flask and the volume was made up to 1000 mL using distilled water.

Buffer (*p*H 7)

The buffer solution (*p*H 7) was prepared by mixing 296.3 mL 0.1 N sodium hydroxide solution with 500 mL 0.1 M monopotassium phosphate solution in a 1000 mL volumetric flask and the volume was made up to 1000 mL using distilled water.

Buffer (*p*H 9)

The buffer solution (*p*H 9) was prepared by mixing 213.0 mL 0.1 N sodium hydroxide solution with 500 mL 0.1 M boric acid in 0.1 M potassium chloride solution in a 1000 mL volumetric flask and the volume was made up to 1000 mL using distilled water.

The buffer solutions were sterilized by passing through 0.2 µm membrane filter, using sterilized glass ware under aseptic condition in a laminar flow chamber. The *p*H of buffer solution was verified using calibrated *p*H meter.

Preparation of Test Substance Stock Solution

A quantity of 41.03 mg imidacloprid (97.5% purity) was weighed and transferred to a 100 mL volumetric flasks (sterilized) containing 5 mL distilled water and volume was made up to the mark with distilled water.

Preparation of Sample Solutions

The sample solutions for *p*H 4.0, 7.0 and 9.0 were prepared by adding 0.5 mL **Stock Solution** in separate 50 mL volumetric flasks containing 5 mL sterilized buffer

solutions of *p*H 4.0, 7.0 and 9.0, respectively. The volume was made up to 50 mL with sterilized buffer solution of *p*H 4.0, 7.0 and 9.0 in duplicate. The headspace of the flask was flushed with nitrogen to remove any residual oxygen. The test vessels were sealed with sterilized tube and polyurethane foam. All the operations were performed under aseptic condition in a laminar flow chamber. The test solutions concentration was 4.0 $\mu g\ mL^{-1}$ (imidacloprid a.i. content).

Incubation and Sampling

The buffered test solutions were incubated in a temperature controlled water bath maintained at 50 ± 0.5 °C in dark. The test samples were analysed for imidacloprid a.i. content at 0 h, 2 h, 4 h and on 5th day. A separate set of six (2 replications for each *p*H) flasks with test solution were maintained at 50 ± 0.5 °C for 5 days, for sterility and *p*H check at the end of the preliminary test.

Determination of *p*H

The *p*H of the samples was determined on the 0 and 5th day using calibrated *p*H analyzer.

Monitoring of Sterility

Spread plate counting method was followed for monitoring the sterility of the samples on 5th day. Readymade nutrient agar media [composition : peptic digest of animal tissue (5.00 g/L), sodium chloride (5.00 g/L), beef extract (1.50 g/L), yeast extract (1.50 g/L) and agar (15.00 g/L)] was used. The media was prepared by suspending 4.2 g nutrient agar in 150 mL distilled water.

The petri-plates, nutrient agar media, and glassware were sterilized by autoclaving at 121 ºC and 1.05 kg/cm^2 pressure for 15 minutes.

The sterilized nutrient agar media was poured into the petri-plates and allowed to solidify. Then three drops of test solution taken from the respective flask using sterilized pipette was added to the surface of the solidified media and spread with sterilized glass spreader. The plates were incubated at 37 ± 2 ºC in BOD incubator. The observations were recorded at 24-hours interval up to 48 hours.

Analysis of Samples by HPLC

All the test samples were analysed by HPLC using the validated analytical method. The validation data and instrument parameters discussed in **chapter-2.**

Results and Discussion

The analytical method was validated before initiating the analysis of imidacloprid samples. The validation covered the aspects namely; (i) linear dynamic range (LDR), (ii) limit of detection (LOD), (iii) limit of quantitation (LOQ), (iv) precision (% RSD) and (v) accuracy (% Recovery). The correlation coefficient for linearity for concentration range 1.0 to 8.0 µg mL^{-1} was found to be 0.999 (**figures 1)**. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.03 and 0.05 µg mL^{-1}, respectively, (Prakash S. *et. al.* 2011). . The precisions (% RSD) were 1.41, 1.39 and 0.40% and accuracies (% Recovery) were 98.58, 99.75 and 100.42% for buffer solution of *p*H 4.0, 7.0 and 9.0, respectively.

Preliminary Test

In the preliminary test, the initial concentration of imidacloprid (0 hour) in buffer solutions at *p*H 4.0, 7.0 and 9.0 was 3.93, 3.89 and 3.99 µg mL^{-1}, respectively (Table 1). The imidacloprid concentration after 2 hours of incubation at 50 ± 0.5 ºC in buffer solutions at *p*H 4.0, 7.0 and 9.0, was 3.94, 3.86 and 3.95 µg mL^{-1}, respectively. The imidacloprid concentration after 4 hours of incubation at 50 ± 0.5 ºC in buffer solutions of *p*H 4.0, 7.0 and 9.0, was 3.81, 3.80 and 3.93 µg mL^{-1}, respectively. The corresponding values after 5 days were 3.73, 3.75 and 3.81 µg mL^{-1}, respectively.

The hydrolysis of imidacloprid at *p*H 4.0 after 2, 4 hours and 5 days of incubation at 50 ± 0.5 ºC was 0.0, 3.05 and 5.09%, respectively. The imidacloprid hydrolysis at *p*H 7.0 after 2, 4 hours and 5 days of incubation was 0.77, 2.31 and 3.60%, respectively. The imidacloprid hydrolysis at *p*H 9.0 after 2, 4 hours and 5 days of incubation was 1.00, 1.50 and 4.51%, respectively.

The per cent hydrolysis data revealed that degradation of imidacloprid at *p*H 4.0, 7.0 and 9.0 was less than 10% in the preliminary test (5days) and therefore no further test was performed. Hence, it is concluded that the theoretical half-life of imidacloprid TC is >1

year at 50 ± 0.5 ºC and imidacloprid is hydrolytically stable in water in abiotic conditions.

Kinetics and Half-lifein abiotic conditions

The hydrolysis of imidacloprid at *p*H 4.0 after 2, 4 hours and 5 days of incubation at 50 ± 0.5 ºC was 0.0, 3.05 and 5.09%, respectively. The imidacloprid hydrolysis at *p*H 7.0 after 2, 4 hours and 5 days of incubation was 0.77, 2.31 and 3.60%, respectively. The imidacloprid hydrolysis at *p*H 9.0 after 2, 4 hours and 5 days of incubation was 1.00, 1.50 and 4.51%, respectively. Similar results have also been reported (Suparna Pal *et. al*). The dissipation was faster under alkaline condition followed by acidic and neutral.

***p*H and Sterility**

The *p*H of the test solutions was within the allowable limit (4.09 to 4.07 for *p*H 4.0; 7.00 to 7.03 for *p*H 7.0 and 9.04 to 9.05 for *p*H 9.0 samples) throughout the study and there was no microbial contamination in the test samples at the end of the preliminary study **(Table 2).**

Results and Discussion

This study was conducted to determine the hydrolysis rate and half-life of imidacloprid technical in buffer solution of *p*H 4.0, 7.0 and 9.0 at 50 ± 0.5 ºC. In the preliminary test, the initial concentration of Imidacloprid (0 hour) in buffer solutions at *p*H 4.0, 7.0 and 9.0 was 3.93, 3.89 and 3.99 µg mL^{-1}, respectively. The imidacloprid concentration after 2 hours of incubation at 50 ± 0.5 ºC in buffer solutions of *p*H 4.0, 7.0 and 9.0, was 3.94, 3.86 and 3.95 µg mL^{-1}, respectively, as shown in **table 1**. The per cent hydrolysis data revealed that degradation of imidacloprid at *p*H 4.0, 7.0 and 9.0 was less than 10% in the preliminary test (5 days).

Conclusion

The hydrolytic degradation of imidacloprid through hydrolysis after 5 days of incubation at 50 ± 0.5 ºC at *p*H 4.0, 7.0 and 9.0 was less than 10%. Therefore, it is concluded that the theoretical half-life imidacloprid is >1 year at 50 ± 0.5 ºC and imidacloprid is hydrolytically stable under abiotic conditions. The imidacloprid is hydrolytically stable under acidic, neutral conditions (*p*H 4.0, 7.0) and hydrolyses in basic condition at *p*H 9.0.

TABLE 1 Data of Imidacloprid Hydrolysis at 50 ± 0.5 ºC in *p*H 4.0, 7.0 and 9.0 Buffer Solutions.

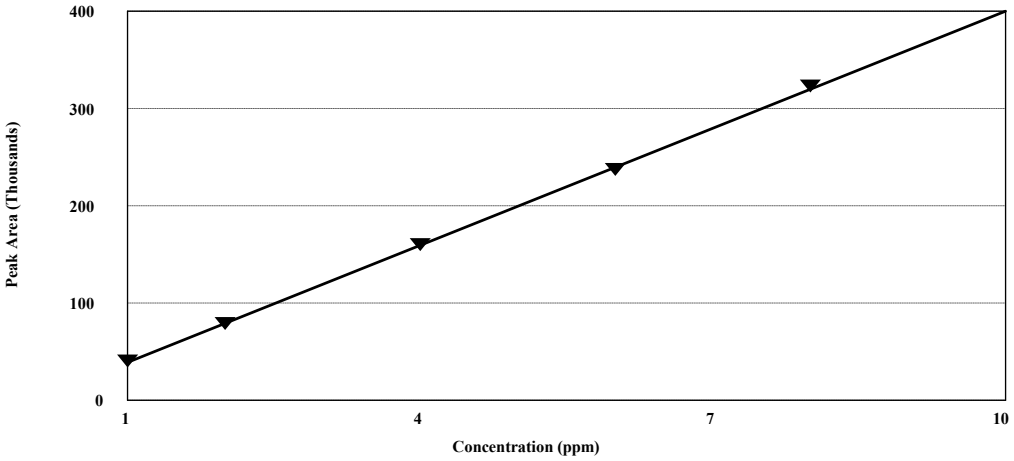
Sampling Interval (Hour/Days)	Test Concentration of Imidacloprid (µg mL^{-1})	Buffer Solution (*p*H)	Imidacloprid Concentration (µg mL^{-1})	Hydrolysis (%)
0 hour	4.00	4.0	3.93	-
		7.0	3.89	-
		9.0	3.99	-
2 hours	4.00	4.0	3.94	0.00
		7.0	3.86	0.77
		9.0	3.95	1.00
4 hours	4.00	4.0	3.85	2.78
		7.0	3.80	2.31
		9.0	3.92	4.50
5 days	4.00	4.0	3.73	4.09
		7.0	3.75	3.80
		9.0	3.51	5.51

TABLE 2 Records of Sterility Test (at 37 ± 0.5 ºC) and *p*H Data

*p*H	Replication	Sampling Interval		Microbial Counts	
		0 hour	Day 5	24 hour	48 hour
4.0	I	4.09	4.07	–	–
	II	4.09	4.08	–	–
7.0	I	7.00	7.01	–	–
	II	7.01	7.03	–	–
9.0	I	9.05	9.05	–	–
	II	9.05	9.04	–	–

Note : – = no microbial growth

Figure 1 Linear Dynamic Curve of Imidacloprid Standard



Slope of the line (a) = -1279.14
Intercept with y -axis (b) = 40260.43
Correlation co-efficient or 'r' value = 0.999

Equation : Y = a + bX

Section II: Biotic Degradation of Imidacloprid

Experimental setup

Biotic degradation of Imidacloprid in Water at *p*H 4.0, 7.0 and 9.0 under Laboratory Conditions

The experiments were carried out under laboratory conditions at 25 ± 2 °C for residue and persistence study of imidacloprid in water. A volume of 25 mL buffer solution was transferred into 50 mL capacity test tube and fortified at three levels 1, 2 and 4 **µg mL^{-1}** with three replications by adding reference standard of imidacloprid, separately. Buffer solutions (*p*H 4.0, 7.0 & 9.0) were fortified and kept in laboratory conditions. During entire study period, test tubes were covered with polythene sheets with few holes and stored at 25 ± 2 °C. The periodic samples were drawn at intervals of 0, 1, 3, 15, 30, 45, 60, and 90 days after application. The samples were drawn in three replication of each treatment and along with control.

Results and Discussion

Imidacloprid dissipation in biotic conditions in soil: The dissipation data of imidacloprid in water are depicted in **table 3**. The dissipation data of imidacloprid in water was show that the degradation of imidacloprid increases with date after application. The % dissipation of imidacloprid water treated @ **µg mL^{-1}** was 25.65, 17.65, and 28.51% after 15 days of application in ***p*H 4.0, 7.0 and 9.0** respectively, and the corresponding value @ 2 and 4 **µg mL^{-1}** was 24.12, 14.65, 27.7% and 22.34, 12.98, and 25.10 respectively. The degradation of imidacloprid was found to be higher in basic conditions (72.54%) than at *p*H 4.0 and *p*H 7.0 after 90 days after application (figures 2 to 4). Shortest half life of imidacloprid was found to be 44 days in basic condition in biotic condition as depicted by the data in **table 4**.

Conclusion

The lowest degradation rate of imidacloprid was 52.45% at *p*H 7.0 in biotic condition. Corresponding value for *p*H 4 and *p*H 9 were 63.28 and 69.43@ 2.0 µg mL^{-1}. The imidacloprid degradation higher in *p*H 9 72.54% @ 2.0 µg mL^{-1}. The degradation of imidacloprid at *p*H 7.0 and *p*H 4 less than *p*H 9, it means imidacloprid stable in neutral and acidic water *p*H. Imidacloprid maximum degradation was found at *p*H 9.0 in basic condition

Table 3 Degradation of Imidacloprid in Water at *p*H 4.0 *p*H 7.0 and *p*H 9.0 in Biotic Conditions

DAS	***p*H 4.0**			***p*H 7.0**			***p*H 9.0**		
	1.0 µg mL^{-1}	2.0 µg mL^{-1}	4.0 µg mL^{-1}	1.0 µg mL^{-1}	2.0 µg mL^{-1}	4.0 µg mL^{-1}	1.0 µg mL^{-1}	2.0 µg mL^{-1}	4.0 µg mL^{-1}
1	5.20	3.98	3.20	4.99	3.24	2.29	6.79	4.56	4.22
3	12.56	14.21	9.86	9.98	7.10	5.56	13.75	11.15	10.91
7	18.34	16.43	14.89	14.78	9.98	7.65	22.54	20.12	19.40
15	25.65	24.12	22.34	17.65	14.64	12.98	28.51	27.6 0	25.10
30	32.65	28.54	24.56	24.56	22.24	18.54	38.86	37.36	32.88
45	46.89	43.67	40.98	36.68	32.67	28.96	50.49	49.45	45.18
60	55.34	53.24	49.90	45.78	42.67	39.76	62.45	60.65	60.37
90	65.57	63.28	58.65	54.12	52.45	50.89	72.54	69.43	63.66

Table 4 Half life of Imidacloprid in Water at *p*H 4.0, *p*H 7.0 and *p*H 9.0 in Biotic Conditions

Water buffer	1.0 µg mL^{-1}	2.0 µg mL^{-1}	4.0 µg mL^{-1}	Mean
***p*H 4**	47.00	55.00	60.00	**54.00**
***p*H 7**	67.00	65.00	60.00	**64.00**
***p*H 9**	44.00	45.00	52.00	**47.00**
Mean	**52.67**	**55.00**	**57.33**	**55.00**

Figure 2 Degradation of Imidacloprid in Water at *p*H 4.0 in Biotic Conditions

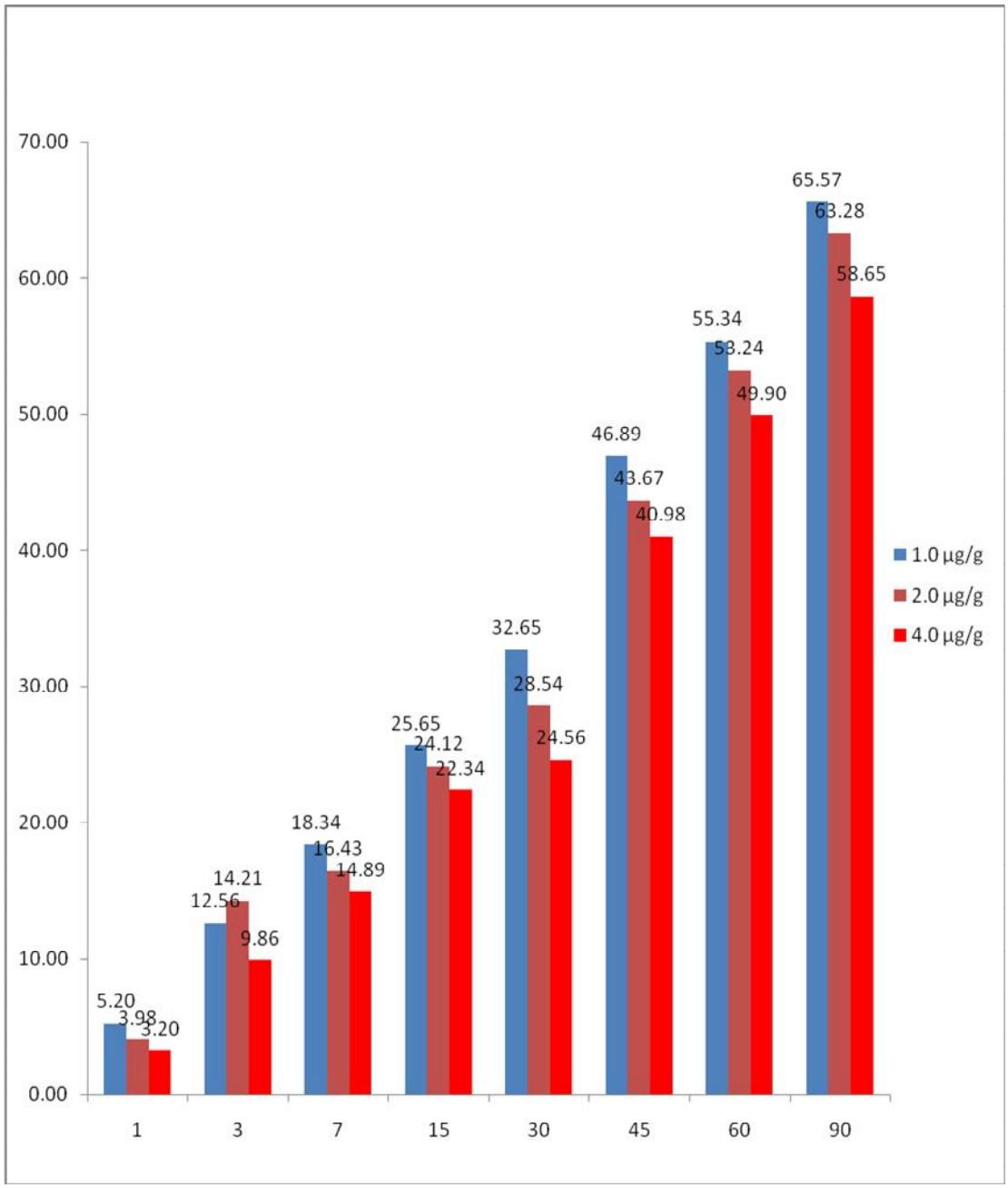


Figure 3 Degradation of Imidacloprid in Water at *p*H 7.0 in Biotic Conditions

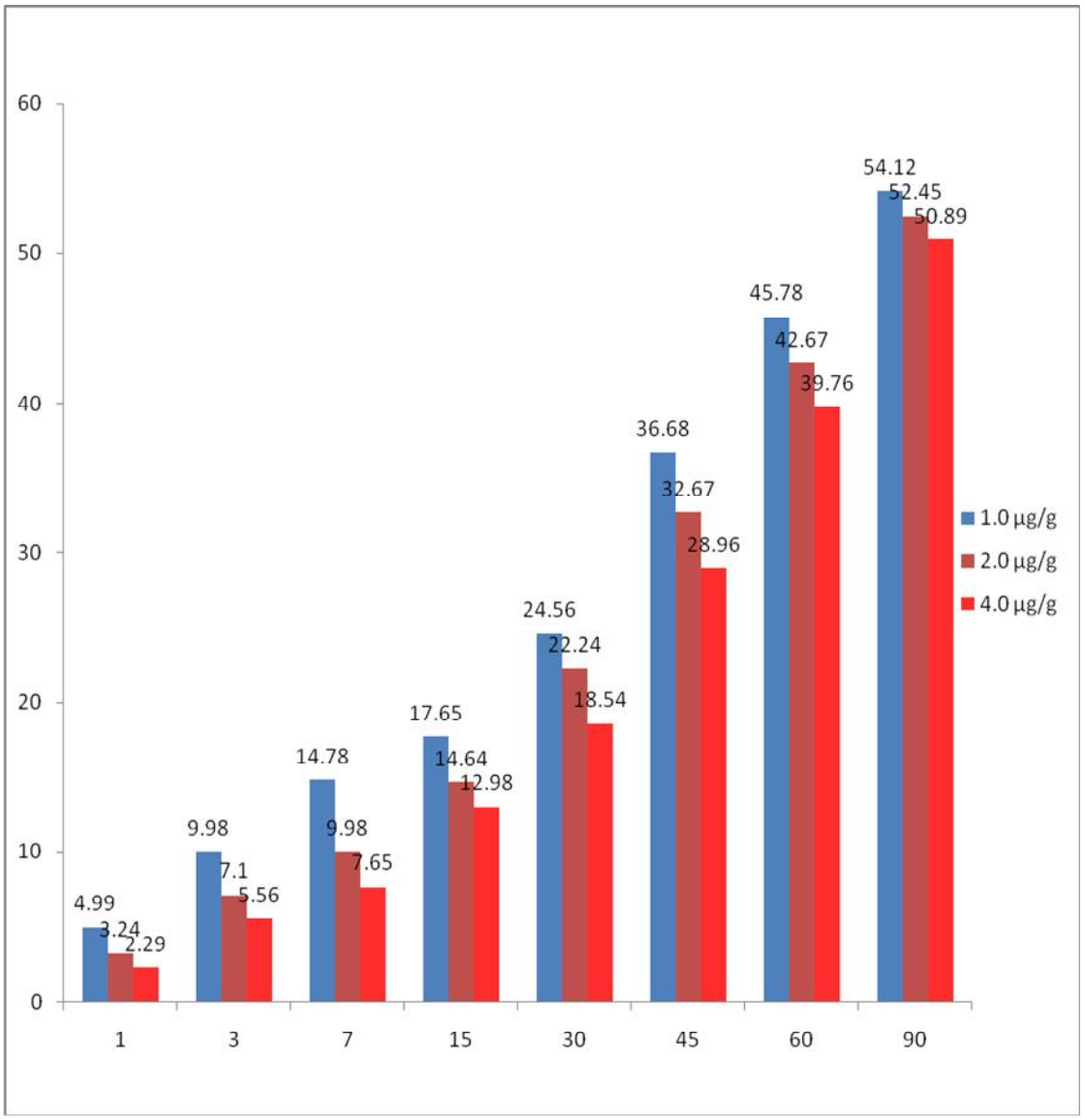
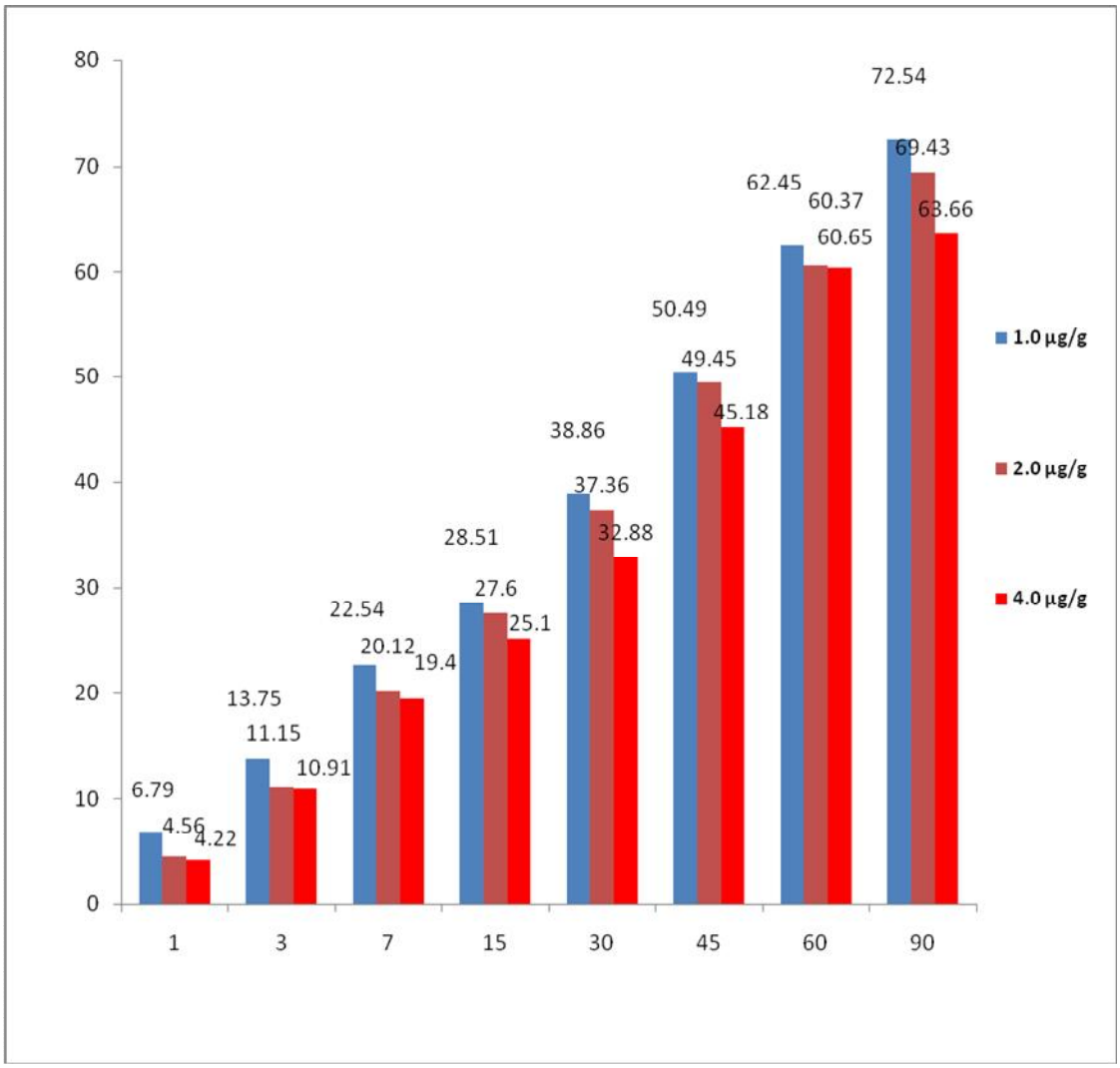

60
50
40
30
20
10
0
4.99
3.24
2.29
9.98
7.1
5.56
14.78
9.98
7.65
17.65
14.64
12.98
24.56
22.24
18.54
36.68
32.67
28.96
45.78
42.67
39.76
54.12
52.45
50.89
1
3
7
15
30
45
60
90
1.0 µg/g
2.0 µg/g
4.0 µg/g

Figure 4 Degradation of Imidacloprid in Water at *p*H 9.0 in Biotic Conditions



.

References

EC, 1997: EC C.7, (1997), "Degradation – Abiotic Degradation Hydrolysis as a Function of *p*H". The European Commission - Classification, Packaging and Labelling of Dangerous Substances in the European Union, Part 2 – Testing Methods.

Tisler T, Jemec A, Mozetic B, Trebse P., (2009), Hazard identification of imidacloprid to aquatic environment. ChemospHere. 76 (7), 907-14.

Suparna Pal, Surajit Dutta, Pramanik S K, Manojit Ghosh, Anjan Bhattacharyya, (2006), Effect of pH on dissipation of pyrazosulfuron-ethyl in water. Pesticide Research Journal, 18, (1),98-100.

Zheng W, Liu W, (1999), Kinetic and mechanism of the hydrolysis of imidacloprid. J. Pesticides Sci. (55), 182-485.