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Research Article

INVESTIGATIONS ON THE PHOTOLYSIS OF IMAZETHAPYR IN AQUEOUS SOLUTIONS UNDER DIRECT SUNLIGHT BY HPLC UV AND LC-MS/MS-ESI - APPLICATIONS OF GREEN ALGA AS A POTENTIAL BIOMARKER

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ABSTRACT

Photolysis of Imazethapyr was studied in Milli-Q water, in acidic (pH 5.0), neutral (pH 7.0) and basic (pH 7.0) buffer solutions at two concentration levels 1.0 µg/ml and 2.0 µg/ml under direct sunlight. Aliquots of water samples were collected, filtered and analysed by HPLC-UV detection at different predetermined intervals. The results showed a limit of quantification (LOQ) 0.01 µg/ml and the recovery was 96 to 105%. The degradation of Imazethapyr under direct sunlight was considerably more rapid than hydrolysis in dark with a half-life of 2.2, 2.4 and 2.6 days for acidic, neutral and basic buffer solutions respectively. The estimated DT_{50} value of Imazethapyr in hydrolysis study was 22.7 days in acidic, 24.5 days in neutral and 26.4 days in basic buffer solutions. The dissipation followed first order kinetics. The influence of cations - Fe^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, $Zn^{2+}$and anions - SO_4^{2-}, Cl^-, ClO_4^-, CO_3^-, HCO_3^- and aeration of oxygen was studied. Major photo transformation products were identified in water using liquid chromatography electrospray tandem mass spectrometry (LC-MS/MS-ESI). The data was compared with similar hydrolysis study conducted at 25°C. The impact of residues if any, present in the aqueous samples after the quantitative indication of residues to below the detectable level was studied by alga bioassay test. Residues dissipated to below detectable level on 15th day. The growth inhibition at this occasion was 65%. Observations on 40th day showed no sign of inhibition in the growth of green alga.

Keywords: Imazethapyr, Photolysis, LC-MS/MS-ESI, Residues, Alga inhibition.

INTRODUCTION

Contamination of water due to the continuous use of crop protection chemicals in agriculture poses a serious environmental problem. However, in aqueous media, many of these chemicals are likely to undergo photochemical transformations under direct sunlight. Imazethapyr [(RS)-5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid] is a pre and post-emergence herbicide used to control the grasses and broad leaved weeds in pulses and oil seeds. Photolysis and hydrolysis are the two main degradation pathways of herbicides in the environment[1]. The abiotic degradation (photo degradation and hydrolysis) of the imidazolinone herbicides - imazapyr, imazethapyr and imazaquin were investigated in water and soil. Hydrolysis and photo degradation followed first-order kinetics for all herbicides[2]. The photodegradation of imazethapyr in pH 7.0 aqueous solution in presence of titanium dioxide and humic acids at different ratios increases the degradation rate due to the presence of semiconductor photo catalyst and humic acids[3]. A capillary electrophoresis method was used to quantify the residues of imidazolinone herbicides; imazapyr, imazamox, imazapic, imazethapyr, imazaquin, and imazamethabenz[4] in water and is subsequently confirmed by liquid chromatography-electrospray ionization mass spectrometry[5].

The selective degradation of enantiomers of the three imidazolinone herbicides, imazapyr, imazethapyr and imazaquin were determined in a variety of soils. The R(+) enantiomer of all the three herbicides has greater herbicidal activity than the less active S(-) enantiomer[6]. Enantiomers of imidazolinone herbicides were resolved using reverse-phase and normal-phase high-performance liquid chromatography with polysaccharide-type chiral columns[7]. The photolysis of Imazethapyr in three paddy water samples was evaluated under laboratory conditions. Imazethapyr is susceptible to both direct and indirect photolysis reactions in water and paddy[8]. Soil pH influenced the Imazethapyr sorption-desorption, which in turn had affected the persistence and bioavailability of Imazethapyr at low pH than at high pH [9-10]. Sulfonylurea, sulfonamide and imidazolinone herbicides were also analyzed in rivers, reservoirs and ground water in the Midwestern United States using LC-MS/MS. At least one of the herbicide was detected above the MRL in 83% of one thirty stream samples, 24% of twenty five ground water samples and 86% of seven reservoir samples[11]. The photocatalytic degradation of Imazethapyr was determined in aqueous suspensions using titanium dioxide as a catalyst. The effect of catalyst loading, initial concentration of Imazethapyr, hydrogen peroxide, pH value and temperature were investigated. It was found that the addition of hydrogen peroxide to the TiO_2 suspension enhanced the degradation rate up to 5.0×10^{-3} mol/L but decreased at higher concentration[12]. The imidazolinone herbicides imazapyr, *m*-imazamethabenz, *p*-imazamethabenz, *m,p*-imazamethabenz-methyl, Imazethapyr and imazaquin were quantified in ground, lake and river water using off-line solid-phase extraction with a carbograph-1 cartridge by reverse-phase liquid chromatography using a UV detector (lambda = 240 nm) and soil samples using LC/ESI-MS under selected ion monitoring mode. The limit of detection was established as 0.1 - 0.05 ng/g[13]. The sorption coefficient of eight herbicides (alachlor, amitrole, atrazine, simazine, dicamba, imazamox, imazethapyr and pendimethalin) was determined in seven agricultural soils from Lithuania. The leaching of all herbicides in soils with high clay and low sand contents were predicted to be slow and it was faster in soils with high sand and low organic matter[14-16]. Two extraction methods, supercritical fluid extraction using CO_2 and solid-phase extraction method are used for extracting two imidazolinone herbicides AC263, 222 and Imazethapyr from three soils were alternatives to soxhlet and liquid/liquid extraction[17]. Two-cartridge solid phase extraction methods were developed to pre-concentrate twelve Sulfonylurea, three imidazolinone and one sulfonamide herbicides from water samples. LC-ESI/MS/MS was used to identify and quantify the residues[18].

The photo degradation of metribuzin, atrazine, propazine and prometryne in water was studied. The photoproducts identified were desaminometribuzin, diketometribuzin and desaminodiketometribuzin[19]. The impact of humic substances on decomposition and degradation kinetics was determined[20]. A study was conducted on the photolysis of trifluralin under sunlight and controlled atmosphere was studied[21]. Hence the reaction rate constants are determined. Photolysis of sulfadiazine[22], atrazine[23], trifluralin[24] in water and manure was studied under simulated sunlight. First order kinetics is followed in aqueous solution. The phototransformation of the herbicide fluometuron in natural sunlight was investigated in Milli-Q and synthetic water. The degradation followed pseudo-first order kinetics[25]. Photolysis of

metamitron in water and soil were studied, the photodegradation was faster in aqueous solution than in soil[26]. The photochemistry of triadimefon and triadimenol was studied in aqueous solution, methanol/water mixtures, in controlled and natural conditions. The kinetics was faster in water than methanol/water mixtures [27].

From the review of literature, it was observed that the dissipation of Imazethapyr in soil was studied using various techniques. However, the data pertaining to the dissipation of Imazethapyr in aqueous solutions under direct sunlight is sparse. The main objectives of present study are to determine the dissipation of Imazethapyr under direct sunlight and to compare the hydrolytic behavior in dark, in different aqueous systems. It is to investigate the effect of cations, anions and oxygen aeration, to identify the photo transformation products by LC-MS/MS-ESI and to assess the impact of residues on the growth of green alga.

MATERIALS AND METHODS

Chemicals

The Imazethapyr (purity 99.8%) reference analytical standard and the Imazethapyr water dispersible granule formulation (70%WG) commercial grade, obtained from the pesticide market outlet were used. HPLC grade acetonitrile and ortho phosphoric acid were obtained from Merck India Limited. The chemicals supplied by Merck India Limited were ferrous sulfate, copper sulfate, cobalt nitrate, nickel sulfate, manganese sulfate, zinc sulfate, sodium sulfate, sodium chloride, sodium perchlorate, sodium carbonate and sodium bicarbonate. All the chemicals used were analytical reagent grade. Distilled water was purified using a Milli-Q apparatus. (Millipore, Bedford, MA, USA).

Instrumentation

A Shimadzu® prominence High Performance Liquid Chromatograph equipped with Ultra Violet detector was used for the quantification of residues. The wavelength of the detector was 230 nm. The Phenomenex® C_{18} column (4.6 mm i.d. and 250 mm length) was used for chromatography. Acetonitrile, pH 3.0 ortho phosphoric acid buffer solutions (40:60) were used as mobile phase for the separation of Imazethapyr. The flow rate was set as 1.0 ml per minute. The retention time of Imazethapyr was 6.2 minutes.

The active Imazethapyr and its breakdown products were confirmed by a High Capacity Ion Trap (HCT plus) LC-MS/MS system supplied by Bruker Daltonik, GmbH, Germany. Drying gas nitrogen was generated using the Nitrox UHPLCMS nitrogen generator. The nebuliser gas nitrogen flow was fixed at 9 lit/min. MS/MS mode operation was done with helium as collision gas. Capillary voltage of 4.5 kV was used in positive ionization mode. The interface temperature was 340 °C. The scan range was 50 - 400 m/Z. Agilent 1200 HPLC system with Zorbax SB C18 column (5 µm particle size, 4.6 mm i.d., 75 mm length) with gradient elution of 0.25 ml per minute having 0.1% formic acid in acetonitrile as mobile phase A, 0.1% formic acid in Milli-Q water as mobile phase B were used.

Method validation

Specificity, linearity and recovery studies were conducted by injecting the control samples of acidic, neutral, basic buffer solutions, mobile phase, Milli-Q water and acetonitrile. Different known concentrations of linearity solutions 2.0, 1.0, 0.5, 0.1, 0.05, 0.01 and 0.001 µg/ml were prepared by serial dilution method using mobile phase and injected in HPLC-UV. The limit of detection was determined as 0.001 µg/ml based on signal to noise ratio 3:1. A calibration curve was plotted between the peak area and concentration of the Imazethapyr.

Recovery studies in Milli-Q water, acidic, neutral and basic buffer solutions were conducted by fortifying different concentrations of standard solutions (0.01, 0.05 and 0.1 µg/ml) of Imazethapyr in the range of LOQ: 5 x LOQ: 10 x LOQ. For the repeatability analysis, five replicate determinations were made at each concentration level. After fortification of standards, the water samples were mixed thoroughly and analysed using HPLC-UV. The method has a limit of quantification (LOQ) 0.01 µg/ml. The RSD% for each concentration studied was calculated using 'Horwitz equation'.

$$RSD\% < 2^{(1-0.5 \log C)} \times 0.67$$

Where C is the concentration of the analyte expressed in percentage.

Experimental

The photolysis of Imazethapyr in water was investigated at concentrations 1 µg/ml and 2 µg/ml. The study was conducted in Milli-Q water, acidic, neutral and basic buffer solutions. Three replicate determinations were made at each fortification level along with a control sample. Two sets of samples were prepared. One set of sample was exposed to direct sunlight. The photolytic activity was monitored by collecting and analyzing the aliquots of water samples periodically on 0, 1, 3, 5, 7, 10 and 15 days. The day temperature of the water samples during the period varied between 27 to 43 °C. During the exposure, the intensity of the sunlight was also measured. On each sampling occasion, aliquots of collected samples were filtered using 0.2 µm PTFE filter and stored in amber colored vials at <5°C before subjecting to HPLC analysis. The other set of sample was kept in dark at 25°C and the water samples were collected on 0, 1, 3, 7, 15, 20, 30, 40, 50, 60, 80, 100 and 120 days. Sterile water was used to analyze the microbial impact on degradation during hydrolysis.

Influence of cations and anions

In the photolytic degradation process, different concentrations (10^{-1}M, 10^{-2}M and 10^{-3}M) of ions were used for the determination of influence of cations and anions. The cations and anions used in the study were ferrous sulfate (Fe^{2+}), copper sulfate (Cu^{2+}), cobalt nitrate (Co^{2+}), nickel sulfate (Ni^{2+}), manganese sulfate (Mn^{2+}), zinc sulfate (Zn^{2+}), sodium sulfate (SO_4^{2-}), sodium chloride (Cl^-), sodium perchlorate (ClO_4^-), sodium carbonate (CO_3^-) and sodium bicarbonate (HCO_3^-). The solutions were mixed well after the addition of metal ions and kept under direct sunlight. At regular time intervals, aliquots of water samples were collected, centrifuged, filtered with 0.2 µm PTFE filter and analyzed by HPLC-UV.

Influence of oxygen aeration

The effect of oxygen aeration in the photolytic degradation process was analyzed by comparing the aerated system with non -aerated system. In aerated and non-aerated system, the water samples were spiked with formulation and exposed to sunlight. The degradation of residue was analyzed by sampling the aliquots at pre-determined intervals, filtered through 0.2 µm PTFE filter and analyzed using HPLC-UV.

Biological analysis

A study was conducted to determine the effect of residues of Imazethapyr on the growth of green alga, *Pseudokirchneriella subcapitata*. The fresh water green alga *Pseudokirchneriella subcapitata* strain no. SAG 61.81 used in the study was purchased from University of Gottingen, Germany. According to OECD 201 guideline, OECD TG 201 medium was prepared and used to maintain the green alga.

The intensity of light used in the growth chamber was 6000-8000 Lux. Under continuous illumination, the control and treatment flasks were kept in the shaker incubator. The flasks in the shaker incubator were shaken continuously at the speed of 110-120 rotations per minute (RPM). During the study period, all the conical flasks were randomly repositioned daily in the test chamber.

Sample stock solution preparation

The stock solution was prepared by weighing a known amount of Imazethapyr 70% WG formulation in 100 ml volumetric flask. The content was dissolved in Milli-Q water and sonicated for 5 minutes. The flask was allowed to settle at room temperature for an hour and the volume was made up to the mark using Milli-Q water. The working solution in 500 ml volumetric flask was prepared using Milli-Q water and exposed to direct sunlight.

Pre-culture preparation

Pseudokirchneriella subcapitata pre-culture was prepared three days before initiation of the study. The inoculated flasks were kept in shaker incubator at 114 - 118 RPM and maintained with continuous illumination of 6942 - 7012 Lux light intensity at 21.9 to 22.8°C for three days. The culture was examined after three days under the microscope and checked for any abnormality or microbial contamination.

Test procedure

Aliquot of 35 ml was taken from exposed samples and diluted with 315 ml of OECD medium. The final concentration was 1 µg/ml. By using 0.1N NaOH, the initial pH was measured and adjusted to 8.12. The test solution (100 ml) was transferred to 250 ml Erlenmeyer flask. Three replicate determinations were made at 1 µg/ml concentration and six replicates for control. From the pre-culture, 100 µl of *Pseudokirchneriella subcapitata* culture was inoculated in the control and treated flasks to get an initial cell concentration of approximately 1×10^4 cells per ml. By visual counting, the cell counts of *Pseudokirchneriella subcapitata* (cells per ml) were made using an improved Neubauer's Haemocytometer under illumination of the microscope at 24, 48 and 72 hours after inoculation. At test termination, the final pH was recorded.

The yield was calculated using the following formula

$$Y_{i-j} = X_j - X_i$$

Where,

Y_{i-j} = biomass from the start of the test to the end of the test

X_i = biomass (cells per ml) at time i (0 hour)

X_j = biomass (cells per ml) at time j (72 hours)

The percent inhibition of the yield (% I_y) at test concentration was calculated as,

$$\% I_y = \frac{(Y_C - Y_T)}{Y_C} \times 100$$

Where,

% I_y = percent inhibition of yield

Yc = mean value for yield in the control group

Y_T = value for yield for the treatment replicate

Validation of the alga study

Within the 72 hours test period, the biomass in the control flasks should be increased exponentially by a factor of 16 times[28]. The mean coefficient of variation, for section by section specific growth rates in the control must not exceed 35%. The coefficient of variation of average specific growth rate in control replicates must not exceed 7%.

RESULTS AND DISCUSSION

Analytical data - Linearity, Recovery and Repeatability

The method was found to be linear with a correlation coefficient of 0.9999 when tested in the range 0.001 to 2.0 µg/ml. The limit of detection was established as 0.001 µg/ml based on signal to noise ratio 3:1. The LOQ for Imazethapyr was established as 0.01 µg/ml based on the recovery study and signal to noise ratio 10:1. The recovery of Imazethapyr in water ranges from 96 to 105%. Repeatability of the method showed acceptable RSD% which is <21.44% for 0.01 µg/ml, <16.83% for 0.05 µg/ml and <15.16% for 0.1 µg/ml, according to Horwitz equation.

Photolysis

The half-life values of Imazethapyr under direct sunlight were 2.1 days in Milli-Q water, 2.2 days in acidic water, 2.4 days in neutral water and 2.6 days in basic water. Under the influence of sunlight, the dissipation of Imazethapyr in aqueous buffer solutions was presented in Table I. The dissipation curve was presented in Figure 1.

Hydrolysis

The calculated half life values of Imazethapyr under dark in Milli-Q, acidic, neutral and basic water were 21.5, 22.7, 24.5 and 26.4 days respectively indicating the stability of the active.

Influence of cations and anions

While using the cations and anions in the photolytic degradation process with three different concentrations, the anions (SO_4^{2-}, Cl^-, ClO_4^-, CO_3^- and HCO_3^-) positively enhanced the degradation of residues. When using the cations, the influence on dissipation rate was varied. The presence of iron, copper and zinc were enhancing the degradation rate of Imazethapyr. Negative impact on degradation rate was observed in manganese and cobalt. For nickel, there is no influence on the dissipation rate. The influence of cations and anions were presented in Tables II and III respectively.

Table I: Photolysis of imazethapyr under direct sunlight in Milli Q, acidic, neutral and basic water

Tested dose	Imazethapyr		Tested Dose	Imazethapyr	
	T1 - 1 µg/ml	T2 - 2 µg/ml		T1 - 1 µg/ml	T2 - 2 µg/ml
Sampling occasions (Days)	Dissipation of residues in Milli Q water (µg/ml)		Sampling occasions (Days)	Dissipation of residues in acidic water (µg/ml)	
0	0.95	1.89	0	0.94	1.85
1	0.81	1.58	1	0.83	1.61
3	0.63	1.22	3	0.65	1.28
5	0.49	0.96	5	0.52	0.99
7	0.12	0.25	7	0.14	0.27
10	0.031	0.069	10	0.036	0.079
15	<LOQ	<LOQ	15	<LOQ	<LOQ
Dissipation of residues in neutral water (µg/ml)				Dissipation of residues in basic water (µg/ml)	
0	0.93	1.84	0	0.95	1.86
1	0.85	1.68	1	0.86	1.66
3	0.61	1.20	3	0.65	1.42
5	0.50	0.95	5	0.49	0.99
7	0.15	0.39	7	0.27	0.78
10	0.051	0.099	10	0.052	0.099
15	<LOQ	<LOQ	15	<LOQ	<LOQ

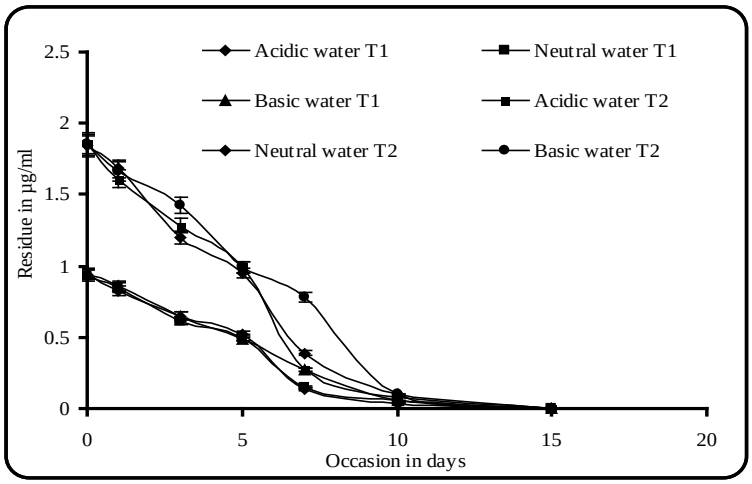

Fig. 1: Dissipation Curve of Imazethapyr under Direct Sunlight

Table II: Influence of cations on the photolysis of imazethapyr

Time (Days)	Cation concentration	Residue (µg/ml)						
		Control	Iron	Copper	Cobalt	Nickel	Manganese	Zinc
0	10^{-1}M	0.95	0.91	0.89	0.99	0.93	0.98	0.88
1		0.81	0.76	0.73	0.87	0.8	0.85	0.71
3		0.63	0.58	0.55	0.69	0.65	0.67	0.56
5		0.49	0.42	0.38	0.56	0.51	0.54	0.40
7		0.12	0.099	0.091	0.42	0.11	0.39	0.084
10		0.031	0.028	0.024	0.23	0.030	0.19	0.026
15		<LOQ	<LOQ	<LOQ	0.109	<LOQ	0.102	<LOQ
0	10^{-2}M	0.95	0.9	0.87	0.98	0.94	0.96	0.86
1		0.81	0.78	0.69	0.86	0.81	0.83	0.7
3		0.63	0.59	0.54	0.7	0.63	0.69	0.54
5		0.49	0.4	0.36	0.53	0.54	0.55	0.36
7		0.12	0.097	0.088	0.4	0.13	0.38	0.081
10		0.031	0.027	0.021	0.19	0.032	0.21	0.022
15		<LOQ	<LOQ	<LOQ	0.082	<LOQ	0.075	<LOQ
0	10^{-3}M	0.95	0.89	0.86	0.97	0.93	0.95	0.84
1		0.81	0.79	0.67	0.85	0.82	0.82	0.71
3		0.63	0.60	0.52	0.68	0.62	0.67	0.52
5		0.49	0.39	0.33	0.52	0.52	0.56	0.33
7		0.12	0.095	0.087	0.41	0.12	0.36	0.079
10		0.031	0.026	0.022	0.21	0.030	0.22	0.021
15		<LOQ	<LOQ	<LOQ	0.060	<LOQ	0.052	<LOQ

Table III: Influence of anions on the photolysis of imazethapyr

Time (Days)	Anion concentration	Residue (µg/ml)					
		Control	Sulfate	Chloride	Perchlorate	Carbonate	Bicarbonate
0	10^{-1}M	0.95	0.87	0.83	0.84	0.83	0.85
1		0.81	0.75	0.76	0.75	0.74	0.77
3		0.63	0.56	0.57	0.54	0.52	0.59
5		0.49	0.4	0.38	0.40	0.41	0.42
7		0.12	0.086	0.084	0.086	0.091	0.090
10		0.031	0.025	0.023	0.024	0.023	0.024
15		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0	10^{-2}M	0.95	0.89	0.85	0.86	0.84	0.87
1		0.81	0.77	0.79	0.78	0.76	0.80
3		0.63	0.57	0.58	0.56	0.55	0.57
5		0.49	0.42	0.4	0.43	0.44	0.44
7		0.12	0.089	0.087	0.091	0.09	0.092
10		0.031	0.027	0.024	0.025	0.024	0.025
15		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0	10^{-3}M	0.95	0.9	0.86	0.88	0.85	0.89
1		0.81	0.78	0.80	0.79	0.80	0.82
3		0.63	0.59	0.60	0.57	0.56	0.59
5		0.49	0.43	0.43	0.46	0.45	0.48
7		0.12	0.096	0.092	0.094	0.091	0.095
10		0.031	0.029	0.026	0.027	0.025	0.028
15		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Influence of oxygen aeration

The degradation of residue was rapid in presence of oxygen aeration. The rate of degradation of Imazethapyr in water was significantly influenced. The residues went to below the limit of determination by 15th day while aerating the water. In the absence of aeration, the degradation was slow and went to below the limit of determination by 25th day. This may be due to enhancement of the amount of dissolved oxygen in water samples have contributed to the formation of oxonium ions facilitating rapid degradation[19].

Dissipation kinetics

The rate constant k was calculated from the dissipation of Imazethapyr with time using the following first order rate equation

$k = \ln a/a\text{-}x/dt$

Where,

dt - Time interval between t_1 and t_2

a, x - Concentration of residues at times t_1 and t_2 respectively.

A graph was plotted between concentrations versus rate. The R^2 value 1.000 indicates first order kinetics in dissipation. The rate constant of Imazethapyr under direct sunlight in two spiked concentrations was 0.489, 0.464 in Milli-Q water; 0.545, 0.523 in acidic water, 0.599, 0.596 in neutral water and 0.588, 0.589 in basic water. The rate constant values for hydrolysis were 0.312, 0.340 in Milli-Q water; 0.287, 0.272 in acidic water, 0.322, 0.349 in neutral water and 0.362, 0.345 in basic water. This clearly indicates that sunlight enhanced the degradation of residues when compared to analysis in dark.

Confirmation of residues and its metabolite

The residues of Imazethapyr and its breakdown products were analyzed using LC-MS/MS-ESI. The herbicide, Imazethapyr got eluted at 15.0 minutes and showed a molecular ion peak at m/Z 290 and the fragment ions appeared at 262, 248 and 177. The breakdown product was eluted at 11.2 to 11.5 minutes. The major break down product identified in the water samples had the molecular ion peak at m/Z 262. The limit of quantification for this method was 0.1 µg/lit. The representative spectra were presented in Figure 2.

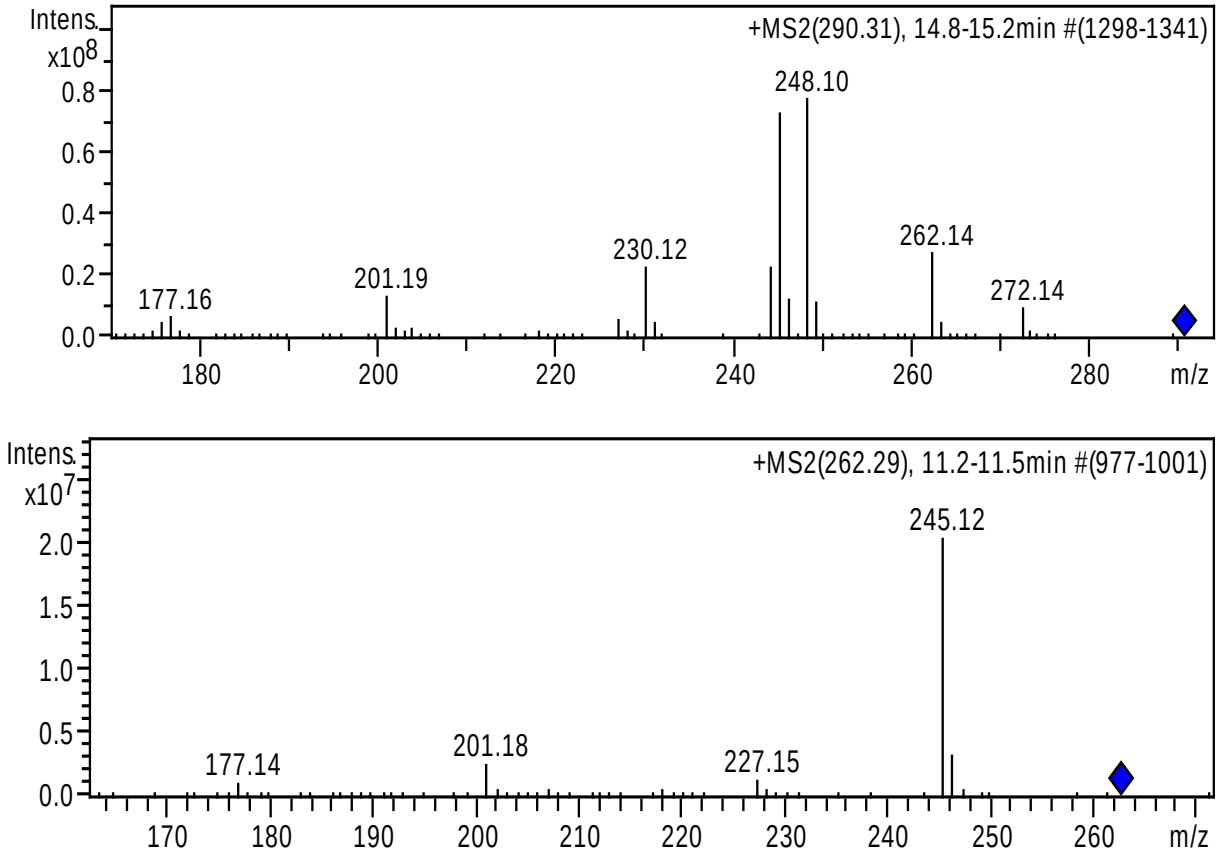

Fig. 2: Representative LC-MS/MS-ESI Spectra of Imazethapyr and its Breakdown Product

Green alga as a biomarker - Impact of residues on alga growth

During the study period, all the flasks were incubated in the shaker incubator. The test conditions viz., temperature, light intensity and RPM of the shaker incubator were recorded daily. The temperature of the medium was 21.2 to 23.1°C, the light intensity was 6785 to 6849 lux and the RPM was 111 – 115.

In the control, the initial cell count was 1×10^4 (10000) cells per ml. The average final cell count (at 72 hours) in the control at 0th, 15th, 25th and 40th days were 159×10^4, 57×10^4, 58×10^4 and 57×10^4 cells per ml respectively. The cells of *Pseudokirchneriella subcapitata* were increased approximately 159, 57, 58 and 57 times in control on 0th, 15th, 25th and 40th day respectively. The percent coefficient of variation (% CV) for section by section specific growth rate in the controls (0-24 hours, 24-48 hours and 48-72 hours) were 10.59%, 24.87%, 24.15% and 22.71% at 0th, 15th, 25th and 40th day respectively (less than 35% as per the OECD guideline). The percent co-efficient of variation of average specific growth rate during the whole test period (0-72 hours) in replicate control flasks was 0.7%, 2.5%, 2.9% and 2.5% at 0th, 15th, 25th and 40th day respectively (less than 7% as per the OECD guideline). These above findings validate the results of the present study.

Initially, (before exposed to sunlight) 84% inhibition was observed at 1.0 µg/ml concentration level. The compound dissipated to below

detectable level at 15th day when analysed by HPLC, whereas 65% inhibition was observed by cell count. On 25th day the inhibition was 16%. The inhibition may be due to the presence of persistent metabolites / breakdown products of Imazethapyr in water samples. This was also confirmed by the LC- MS/MS-ESI analysis. The breakdown product identified on this occasion was 2-[4- methyl-= 5-oxo-4-(propan-2-yl)-4,5-dihydro-1H-imidazol-2-yl]pyridine-3-carboxylic acid and its molecular mass is 261. Analysis of 40th day samples showed no sign of inhibition in the growth of green alga and the growth was on par with the control.

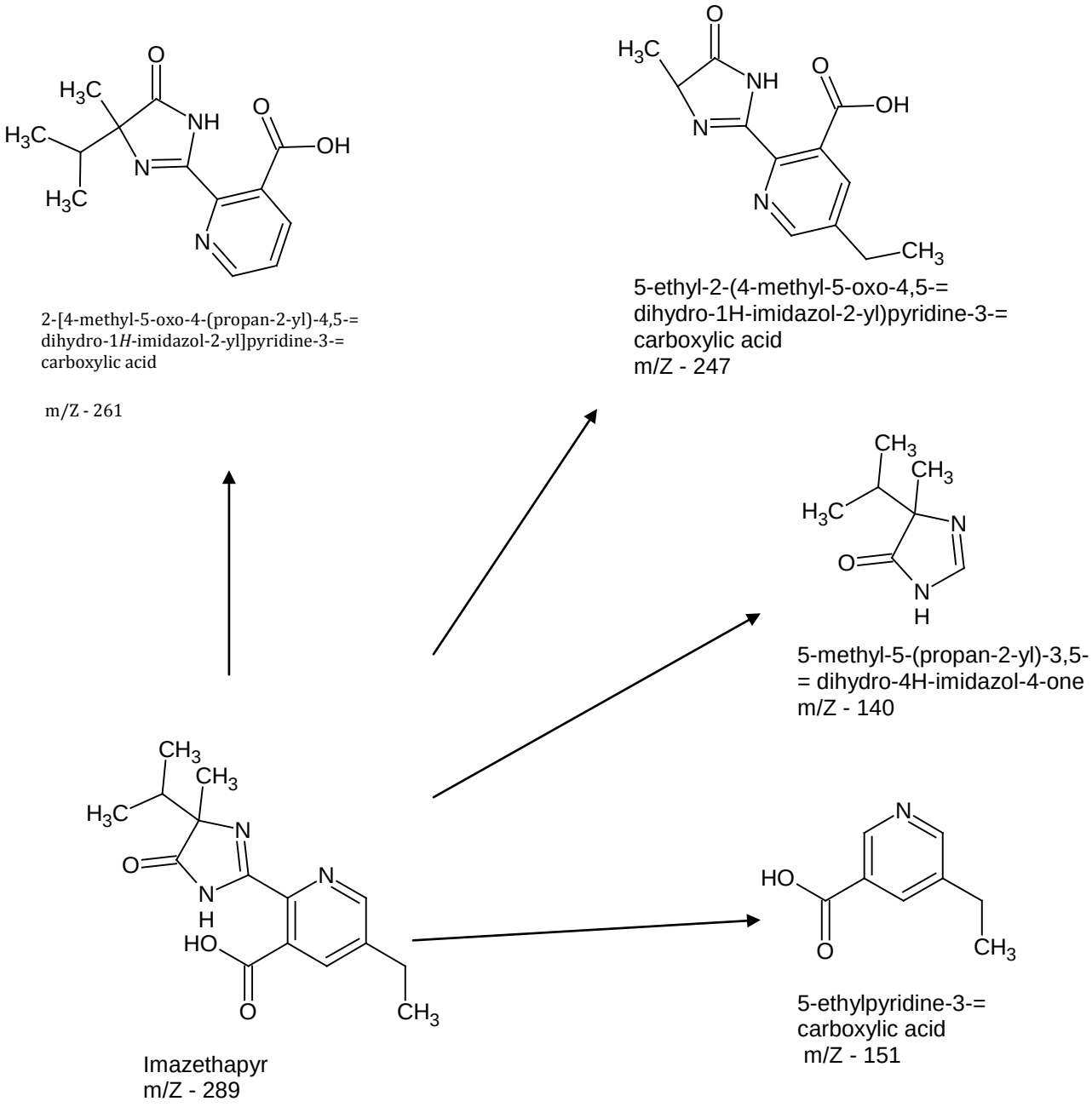

Fig. 3: Photolytic Pathway of Imazethapyr in Water

The degradation pathway of Imazethapyr was presented in Figure 3.

CONCLUSION

Under direct sunlight, the photolysis of Imazethapyr in water was very rapid when compared to hydrolysis study conducted at 25°C. The dissipation of Imazethapyr in water followed first order kinetics. Metabolite of Imazethapyr 2-[4-methyl-5-oxo-4-(propan-2-yl)-4,5-dihydro-= 1H-imidazol-2-yl]pyridine-3-carboxylic acid was identified using the LC-MS/MS-ESI. The rate of degradation was influenced due to presence of cations and anions. The degradation of Imazethapyr was rapid while aerating the water. Under direct sunlight, residues of Imazethapyr dissipated to below detectable level by 15th day but the impact of residues on the growth of green alga indicates the growth inhibition up to 25th day. This may be due to the presence of breakdown product (2-[4-methyl-5-oxo-4-(propan-2-yl)-4,5-dihydro-1H-= imidazol-2-yl]pyridine-3-carboxylic acid) in the water samples. Present investigations successfully highlighted the applications of green alga as a biomarker in identification of potential xenobiotics.

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