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In vitro dissipation of *S*-metolachlor herbicide in different pH water

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Abstract

In vitro dissipation of *S*-metolachlor was studied in neutral, acidic and basic water at 0.2 – 0.4 µg/g (T₁) and 0.4 - 0.8 µg/g (T₂) levels of fortification at All India Network Project on Pesticide Residues, ICAR, Unit-9, Anand Agricultural University, Anand (Gujarat), India. The *S*-metolachlor dissipated much rapidly in acidic and basic water as compared to neutral. Between the acidic and basic water, the rate of degradation was comparatively faster in basic water. In all the three pH water, the degradation followed first order kinetic with mono and biphasic mode. The herbicide underwent degradation at much faster rate in acidic and basic waters as the residue reached below determination level (BDL) of 0.05 µg mL⁻¹ with half-life values ranging from 1.8 to 12.5 day and 12.04 to 301.0 day for Phase-I and Phase-II, respectively. However, in neutral water the rate of degradation was much slower and the residues reached below determination level on the 60th day with half-life ranging 3.4 to 12.5 and 100.3 to 300.1 days for Phase-I and Phase-II, respectively.

Keywords: Dissipation, gas liquid chromatography, *S*-metolachlor, water

Introduction

Water is an essential natural resource required by all living organisms. Unfortunately, aquatic bodies act as sink as contaminated runoff water from treated field flows into it. In India, several workers have reported the contamination of drinking water with pesticides and heavy metals (Kumar *et al.*, 1995) [7]. The present environmental concern is the contamination of aquatic ecosystems due to pesticide discharges from manufacturing plant, agricultural runoff, leaching, accidental spills and other sources. The degradation of synthetic pyrethroids in the environment is an important index in the evaluation of ecological risk of pesticides (Selvam *et al.*, 2013) [11]. Once the pollutants enter into the water bodies like rivers, lakes, ponds, wells and ground water, it may persist and enter food chain by bioaccumulation (Aharanson, 1987) [1]. Persistence of pesticides in water depends on the nature of pesticides, pH, temperature, soil conditions, rainfall and aquatic biota (Di *et al.*, 2002) [3]. Pesticides may have many different fates when released into the environment and understanding their behavior is a major scientific interest. Moreover, the pesticide persistence is decided by the microbiological composition of soil, in particular by the activities of soil enzymes (Gevao *et al.*, 2000) [5]. The information about these processes is important for the evaluation of the dissipation rate of herbicides which allows for selecting the substances which may pose a potential threat to the natural environment as well as human and animal health.

Among herbicides, a very important one is *S*-metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl]aceta-mide) used for selective weed control, with wider use in sorghum, maize, cotton, potato, soybean, peanut or sunflower (Tomlin, 2003) [13]. It is a non-ionic compound belonging to the chloroacetanilide herbicide classified as an inhibitor of very-long-chain fatty acid (VLCFA) formation (Tanetani *et al.*, 2011) [12]. In its commercial formulation, *S*-metolachlor contains 88 per cent *S*-enantiomer and 12 per cent *R*-enantiomer, but the only biological active ingredient is *S*-enantiomer (Dale *et al.*, 2006) [2]. Different behaviours in the soil sorption and dissipation processes are observed for this herbicide, which is related to its different chemical properties. *S*-metolachlor has a relatively high solubility in water (480 mg/L), and it is highly soluble in organic solvents such as acetone, ethyl acetate, toluene and xylene (PPDB, 2014) [9]. Furthermore, its toxicological and environmental profile is favourable for mammals, birds and insects (honeybees included),

except that it can be extremely toxic to fish and aquatic species (Yaw-Jian *et al.*, 1999) [14]. Dissipation study of pesticides is an important tool to determine potential water contamination and verify the actual pesticide concentration in the water to assure long term weed control. Taking this point under consideration, an *in vitro* investigation was carried out to determine the dissipation pattern of *S*-metolachlor in three different pH water *viz.*, 4.0, 7.0 and 9.2. In the aquatic system containing less organic matter, pH is the major environmental factor that influenced insecticide hydrolytic degradation. Present study throws the light on the effect of pH on hydrolytic degradation of *S*-metolachlor in water.

Materials and Methods

Three types of water were used in the present study (pH 4.0, pH 7.0 and pH 9.2) which were prepared using pH buffer capsules procured from E. Merck. The experiment was laid out in chronological randomized design with three replications. The room temperature varied in the range of 21-24 °C and relative humidity was in the range of 39- 80%. Certified reference material of *S*-metolachlor solution in acetone was spiked in three types of water with different pH i.e. acidic (pH 4.0), neutral (pH 7.0) and basic (pH 9.2) @ 0.2-0.4 ppm (T₁) and 0.4-0.8 ppm (T₂). The experiment was performed with 3 replications. A representative 20 ml water sample of pH 4.0, pH 7.0 and pH 9.2 in 50 ml polypropylene tube. Solutions of the herbicide *S*-metolachlor in toluene applied drop wise to the water in each tube to give 0.2-0.4 ppm (lower dose) and 0.4-0.8 (higher dose) ppm. The water was mixed to give a uniform distribution of herbicide. The samples were drawn at 0 (2 hr after application), 1, 3, 5, 10, 20, 30, 40, 50 and 60 days after treatment. The residue of *S*-metolachlor was determined from the samples collected.

Extraction Procedure of *S*-metolachlor from Different Type of Water:

A representative 20 ml *S*-metolachlor fortified water sample of pH 4.0, pH 7.0 and pH 9.2 was taken into 50 ml capacity polypropylene centrifuge tube. To this 8 ml hexane: dichloromethane (40:60) were added for extraction and the tube was shaken vigorously for 2.0 minutes and vortexed for 2.0 minutes and the tube was kept at room temperature for 15 minutes. From this, 2.0 ml aliquot was transferred to 15 ml capacity glass test tube and evaporated to dryness in TurboVap® under nitrogen at 45 °C. Final volume was made to 2 ml with hexane and analyzed on GC using ECD for *S*-metolachlor.

Validation Study: Validation of method was performed with different types of water, in terms of recovery studies, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ). The recovery study was carried out at LOQ, 5 X LOQ and 10 X LOQ levels.

Linearity Study: A linearity study was performed in GLC. For the linearity study, response (height/area) *v/s* concentration was plotted. To establish the linearity seven different concentrations of the standards *viz.*, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 ppm were injected and their response (μV) was recorded. The volume of the standard used for the injection was 1.0 μL. A correlation coefficient and equation was determined by using best fit model of linear relationship.

Limit of Detection (LOD) and Limit of Quantification (LOQ) study: Prior to quantization of herbicide in different

types of water the LOD and LOQ were worked out. This was carried out by injecting matrix-match herbicide to get signal to noise ratio 1:3 for LOD and 1:10 for LOQ.

Recovery Study: Before taking up analysis of test sample for each treatment, control sample were processed along with spiked ones. The samples of different type of water were collected for the recovery study. The samples each of 20 mL were fortified with herbicide at 0.05, 0.25 and 0.5 ppm level of fortification.

Certified reference materials (purity 99.06%) were obtained. All organic solvents used in the standard preparation were of analytical grade. Gas Liquid Chromatograph were used for the qualitative and quantitative estimation of pesticide residues *viz.*, Varian 450 GC equipped with Electron Capture Detector (ECD). The method of calculation of herbicide content for each sample was calculated by using following formula.

$$\text{Herbicide content } (\mu\text{g g}^{-1}) = \frac{A_1}{A_2} \times \frac{V}{W} \times C$$

Where,

A₁ = Peak area of sample

A₂ = Peak area of standard

V = Volume of sample extract

W = Weight of soil sample for extraction

C = Concentration of standard

While studying the degradation pattern of the *S*-metolachlor it was observed that it followed the first order kinetics and mathematically represented as below:

$$Y = A \times e^{-kt}$$

Where,

Y = amount of pesticide at time t, mg/m²

A = initial amount of pesticide, mg/m²

k = dissipation rate constant/days

t = time, days

The data obtained from the study were subjected to regression analysis to determine the dissipation pattern and half- life (DT₅₀). The half- life values of *S*-metolachlor were calculated using the following formula

$$DT_{50} = \frac{\text{Log } 2}{k}$$

Where,

DT₅₀ = half-life of *S*-metolachlor, days

k = dissipation rate constant/days

Results and Discussion

Method validation studies for *S*-metolachlor from different types of water

Linearity study: A linearity study was performed to determine the performance of ECD detector. For the linearity study, a graph of detector's response *vs* concentration was plotted. To establish the linearity of *S*-metolachlor on Gas Liquid Chromatograph equipped with electron capture detector, equal volume of seven different concentrations of *S*-metolachlor *viz.*, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 ppm were injected and their corresponding response were

recorded. The volume of the standard used for the injection was 1.0 μL . Linearity of the detector for *S*-metolachlor was calculated (graphically depicted in Figure 1) by plotting response (area) vs concentration. As per the data obtained in linearity study (Table 1), it was found linear in the range 0.01 to 1.0 ppm and the R^2 values obtained from the correlation equation were calculated by adopting positive linear correlation model ($y = a + bx$) which was >0.99 .

Precision study: Precision represents random errors of a set of replicate measurements. This is calculated as a (relative) standard deviation of replicate measurement. Less precision is reflected by a large standard deviation. Precision depends on the conditions and therefore can be expressed as repeatability and reproducibility. This has nothing to do with true or reference value. Repeatability is a multiple measurements of a sample/standard by the same analyst over a short period of time. In the present study five replicates of 1 ppm was injected and the response as area was recorded as mentioned in Table 2. The RSD (%) was worked out as 0.25 per cent, as measure of instrument precision.

Limit of detection (LOD) and limit of quantification (LOQ) study: The method quantification limit (LOQ) of the weedicide was worked out by injecting the seven different concentrations viz., 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 ppm of the standard prepared in control matrix of soil in GLC (ECD) so as to quantify and detect minimum response and to get repeatable and constant peak area. In this case 0.01 ppm gave the peak area more than 10 times the noise in all the matrices which were considered for LOQ. LOQ for instrument was 0.01 ppm and final sample volume being injected ranged from 0.35- 1.0 g/ml the sample injected in GLC ranged from 0.35- 1.0 mg and therefore, LOQ for method could be 0.03 $\mu\text{g/g}$. However, considering matrix interference due to various type of water an LOQ of 0.05 $\mu\text{g/g}$ was decided to adopt.

Recovery study of *S*-metolachlor for different type of water: Prior to dissipation of *S*-metolachlor, different types of water with pH levels 4.0, 7.0 and 9.2 were subjected to recovery study. Each water type was spiked at 3 different levels i.e. 0.05, 0.25 and 0.5 ppm and further analyzed as per the method of extraction. The data obtained from the study are depicted in Table 3. The recovery experiment was performed with 3 replications along with a control. For the water with pH 4.0, 7.0 and 9.2, *S*-metolachlor was spiked at 0.05, 0.25 and 0.5 ppm and the mean recovery was found in the range of 79.43-90.05, 84.10-99.17 and 79.67-94.50 per cent, respectively. The per cent relative standard deviation within the replicates (RSD_{WR}) obtained for the corresponding water samples and spiking levels were in the range of 1.28-4.67, 0.86-5.13, and 0.51- 4.13, respectively. Similar trends of results were reported by Hajra *et al.* (2015) was showed that average recovery of propaquizafop from water samples of different pH fortified at 0.05, 0.15 and 0.25 $\mu\text{g/ml}$ concentration level were 90.33, 87.0, and 81.22 per cent respectively in acidic, neutral and alkaline water. The present analytical method employed for the extraction of *S*-metolachlor from different pH water was found accurate and precise as mean recovery ranged from 79.43- 99.17 per cent. The RSD_{WR} was in the range of 0.51- 5.13 per cent which was for below 20 per cent which is within the limits prescribed by the SANTE (2017) [10] guidelines. According to the SANTE guidelines, any analytical method which records mean

recovery in the range of 70- 120 per cent and RSD_{WR} below 20 per cent is accurate and precise. Hence, the method employed in the present study for the extraction of *S*-metolachlor from water was accurate and precise.

Dissipation Study of Different Type of Water Acidic water (pH 4.0)

In vitro dissipation of *S*-metolachlor was studied in water with 4.0 pH and it was found that residue persisted till 50th day at lower dose and till 60th day at higher dose. The initial residue levels on 0 day were 0.16 and 0.34 $\mu\text{g/g}$ at lower and higher dose, respectively. The corresponding levels thereafter reached to 0.13 and 0.28 $\mu\text{g/g}$ within 24 hours, indicating 18.75 and 17.65 per cent loss. Thereafter, the lower dose level declined steadily and reached 0.05 $\mu\text{g/g}$ on the 10th day and remained constant till 50th day. For higher dose, the residues persisted till 60th day as the LOQ was 0.05 $\mu\text{g/g}$ (Table: 4). the mode of dissipation was found to be monophasic and biphasic in nature at lower and higher dose, respectively. At lower dose the residue dissipated with half- life of 6.02 days. At higher dose, the rate of dissipation was rapid till 7th day with half- life of 3.16 days during phase- I (0- 7 days). Thereafter, the residue persisted with much slower rate of degradation and half- life of *S*-metolachlor was found to be 150.5 days during 7 to 60 days (phase- II). From 20th to 40th day, decrease was not reported and therefore, persistence revealed much longer (Figure 2).

Neutral water (pH 7.0)

When studied the dissipation of *S*-metolachlor in water with 7.0 pH, it was found that residue persisted till 60th day at both lower and higher dose. The initial residue levels on 0 day were 0.24 and 0.54 $\mu\text{g/g}$ at lower and higher dose, respectively. The corresponding levels thereafter reached to 0.24 and 0.38 $\mu\text{g/g}$ at lower and higher dose, respectively within 24 hours at lower and higher dose, indicating corresponding loss at 0.00 and 29.63 per cent. Thereafter, the levels declined steadily and reached 0.12 $\mu\text{g/g}$ on 40th days at either dose. At lower and higher dose of *S*-metolachlor persisted till 60th day (Table: 4). The mode of dissipation was found to the biphasic in nature for both the dose. At lower dose, the rate of dissipation was rapid till 10th day with half-life of 12.5 days during phase- I (0- 10 days). Thereafter, the residue persisted with much slower rate of degradation and half- life of *S*-metolachlor was found to be 301 days during 10 to 60 days (phase- II). Similar trend was observed even at higher dose, though phase- I of rapid dissipation revealed 0-10 days with half- life of 3.4 days (Figure 3). Thereafter, the residues persisted with half- life of 100.3 days during 10 to 60 days (phase- II).

Basic water (pH 9.2)

When studied the dissipation of *S*-metolachlor in water with 9.2 pH, it was found that residue persisted till 30th and 60th days at lower and higher dose, respectively. The initial residue levels on 0 day were 0.12 and 0.41 $\mu\text{g/g}$ at lower and higher dose, respectively. The corresponding levels thereafter reached to 0.09 and 0.19 $\mu\text{g/g}$ within 24 hours, indicating 25.00 and 53.66 per cent loss. Thereafter, the levels declined steadily and reached 0.05 on 7th day and remained constant till 30th day resulting 58.33 per cent loss at lower dose. However, at higher dose, the level reached to 0.06 $\mu\text{g/g}$ on the 60th day resulting 85.37 per cent loss (Table: 4). The mode of dissipation was found to be monophasic and triphasic at lower and higher dose, respectively (Figure 4). At lower dose, the

rate of dissipation was rapid till 7th day with half- life of 5.9 days during phase- I (0-7 days). Thereafter, the residue, remained constant till 30th day. The rate of dissipation at higher dose was rapid till 3rd day with half- life of 1.8 days during phase- I (0- 3 day). Thereafter, the residue persisted with half- life of 12.04 days for phase- II (3- 10 days) and half- life 150.5 days during phase- III (10- 60 days).

Hazra *et al.* (2015) [6] reported the residues of propaquizafop in water at different pH (4.0, 7.0 and 9.2) at different time intervals and revealed that the initial concentrations (2 hr after application) was 0.87, 0.90 and 0.31 µg/ml for lower dose @ 1 µg/ml and 1.77, 1.78 and 0.48 µg/ml for higher dose @ 2 µg/ml. They reported comparatively higher dissipation was observed in water at pH 4.0 and 9.2 at pH 7.0, especially during the later stage of the experiment. The residue in the reported study dissipated with time and reached below determination level at 7th day of fortification in alkaline water. The dissipation was faster under alkaline and acidic condition followed by neutral pH. The faster dissipation of propaquizafop in water at alkaline and acidic pH could be attributed the fact that alkaline as well as acidic environment favours hydrolytic rate of propaquizafop. Similar trend was reported by Gennari *et al.* (2008) [4] who showed that degradation of triasulfuron in both buffer solution and soil was highly pH-sensitive. The rate of degradation could be described with a pseudo first-order kinetic and was much faster at pH 4 and 9 than at pH 7. Similar trends were reported by Negre *et al.* (1988) [8] who revealed that fluzifop-butyl was stable in sterile buffered water at pH 7, with more than 90% of the initial amount remaining in solution after 21 days. Therefore, it can be concluded that *S*-metolachlor persisted longer in neutral water than acidic and alkaline water. The degradation of *S*-metolachlor in water degraded mainly by hydrolysis. Pesticide contamination of soil and groundwater is a common problem in the environment. In this study, we have examined the pH dependent hydrolytic degradation behavior

of *S*-metolachlor in aqueous media. It is evident from the data obtained in the study that *S*-metolachlor degraded rapidly at acidic and basic pH with respect to neutral condition (graphically depicted in Figure 5 and 6). Chemically, *S*-metolachlor is a chloroacetyl and therefore it is prone to hydrolysis. Nonetheless, abiotic hydrolysis in different pH is unlikely due to low solubility of *S*-metolachlor in water.

Table 1: Response of *S*-metolachlor on ECD at different concentrations

Concentration (ppm)	Detector response (Area) µV
0.01	36.8
0.025	104.7
0.05	176.1
0.10	334.7
0.25	782.0
0.50	1292.7
1.00	2373.5

Table 2: Response of *S*-metolachlor on ECD at 1.00 ppm

Replicate	R-I	R-II	R-III	R-IV	R-V	Average	SD	% RSD
Area (µV)	2514.4	2527.7	2522.4	2530.1	2526.8	2524.28	6.19	0.25

Table 3: Recovery of *S*-metolachlor in water with different pH

pH level	Fortification level (µg/g)	Mean recovery (%) n = 3	Standard deviation (±SD)	% RSD
4.0	0.05	79.43	3.71	4.67
	0.25	82.52	1.06	1.28
	0.50	90.05	1.28	1.42
7.0	0.05	84.10	0.81	0.86
	0.25	99.17	4.09	5.13
	0.50	86.35	2.58	2.98
9.2	0.05	94.50	0.81	0.86
	0.25	79.67	3.09	4.13
	0.50	80.10	0.41	0.51

Table 4: Dissipation of *S*-metolachlor in water at different pH water

Days	Residues (µg mL ⁻¹) * ± SD					
	Acidic water (pH 4.0)		Neutral water (pH 7.0)		Basic water (pH 9.2)	
	T ₁	T ₂	T ₁	T ₂	T ₁	T ₂
0	0.16 (±0.024)	0.34 (±0.034)	0.24 (±0.020)	0.54 (±0.095)	0.12 (±0.016)	0.41 (±0.040)
1	0.13 (±0.024)	0.28 (±0.037)	0.24 (±0.035)	0.38 (±0.061)	0.09 (±0.005)	0.19 (±0.020)
3	0.09 (±0.030)	0.10 (±0.070)	0.17 (±0.013)	0.25 (±0.039)	0.08 (±0.012)	0.12 (±0.018)
5	0.08 (±0.060)	0.09 (±0.030)	0.17 (±0.027)	0.19 (±0.028)	0.06 (±0.010)	0.11 (±0.010)
7	0.06 (±0.090)	0.08 (±0.003)	0.15 (±0.018)	0.15 (±0.019)	0.05 (±0.003)	0.10 (±0.011)
10	0.05 (±0.005)	0.08 (±0.002)	0.14 (±0.014)	0.15 (±0.025)	<LOQ	0.08 (±0.013)
20	<LOQ	0.07 (±0.008)	0.13 (±0.030)	0.15 (±0.021)	<LOQ	0.08 (±0.003)
30	<LOQ	0.07 (±0.009)	0.13 (±0.016)	0.12 (±0.003)	<LOQ	0.08 (±0.002)
40	<LOQ	0.07 (±0.012)	0.12 (±0.018)	0.12 (±0.003)	<LOQ	0.08 (±0.013)
50	<LOQ	0.06 (±0.005)	0.12 (±0.021)	0.11 (±0.015)	<LOQ	0.07 (±0.012)
60	<LOQ	0.06 (±0.006)	0.11 (±0.016)	0.10 (±0.019)	<LOQ	0.06 (±0.003)
Phase I	0-10 days	0-7 days	0-10 days	0-10 days	0- 7 days	0- 3 days
Regression equation	y=1.158-0.05x	y=1.473-0.095x	y=1.3648-0.0248x	y=1.696-0.088x	y=1.046-0.051x	y=1.545-0.166x
Correlation coefficient	0.958	0.846	0.8687	0.968	0.964	0.892
(T _{1/2}) days	6.02	3.16	12.5	3.4	5.9	1.8
Phase II	--	7-60 days	10-60 day	10-60 days	--	3-10 days
Regression equation	--	y=0.917-0.002x	y=1.1619-0.0019x	y=1.212-0.003x	--	y=1.163-0.025x
Correlation coefficient	--	0.894	0.936	0.932	--	0.981
(T _{1/2}) days	--	150.5	301	100.3	--	12.04
Phase III	--	--	--	--	--	10-60 days
Regression equation	--	--	--	--	--	y=0.952-0.002x
Correlation coefficient	--	--	--	--	--	0.680
(T _{1/2}) days	--	--	--	--	--	150.5

*Mean of three replicates; LOQ= Limit of quantification (0.05 µg mL⁻¹); figure in parentheses denotes % dissipation

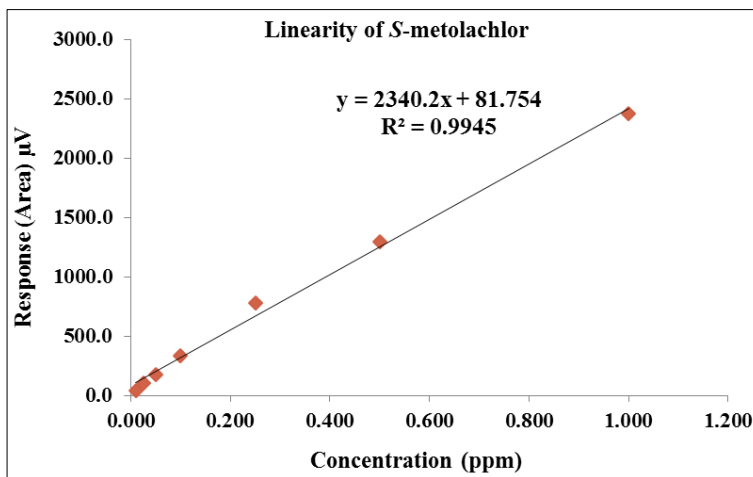


Fig 1: Linearity of S-metolachlor

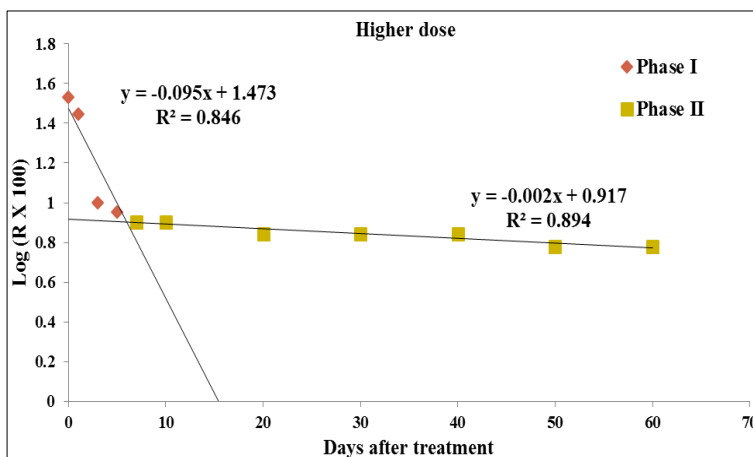
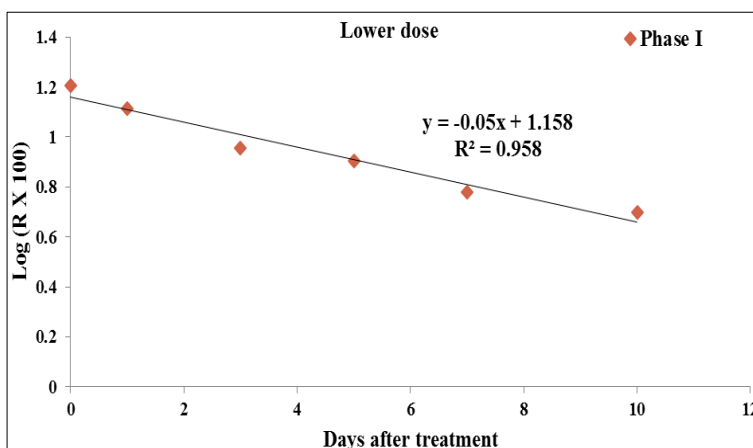
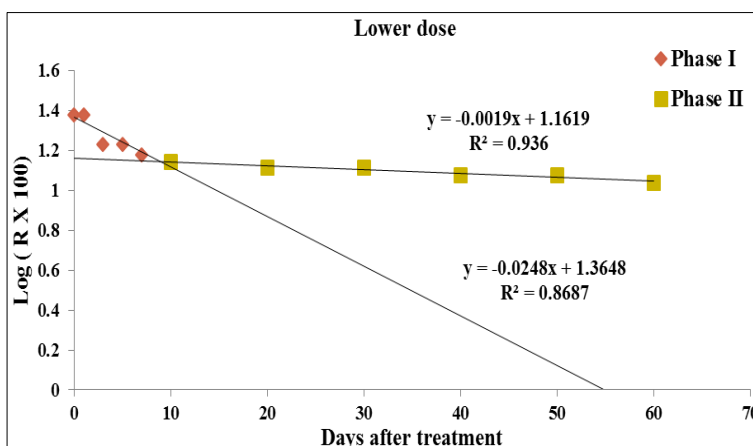


Fig 2: *In vitro* dissipation pattern of S-metolachlor at lower and higher dose in 4.0 pH water



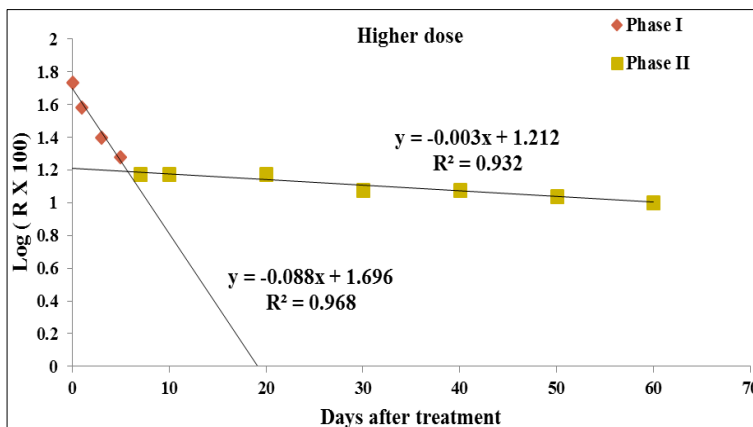


Fig 3: *In vitro* dissipation pattern of *S*-metolachlor at lower and higher dose in 7.0 pH water

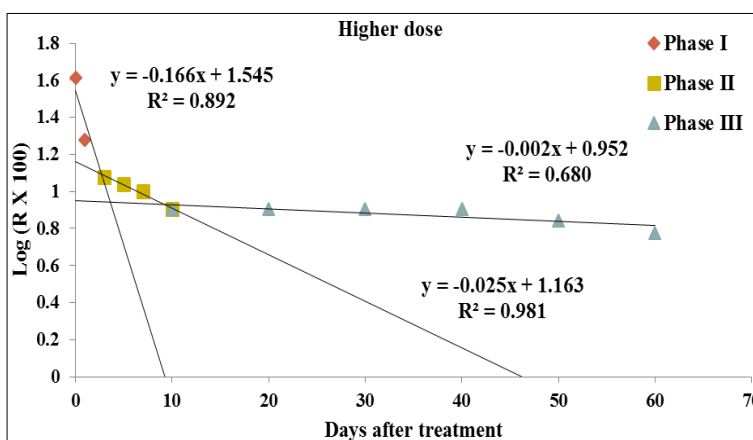
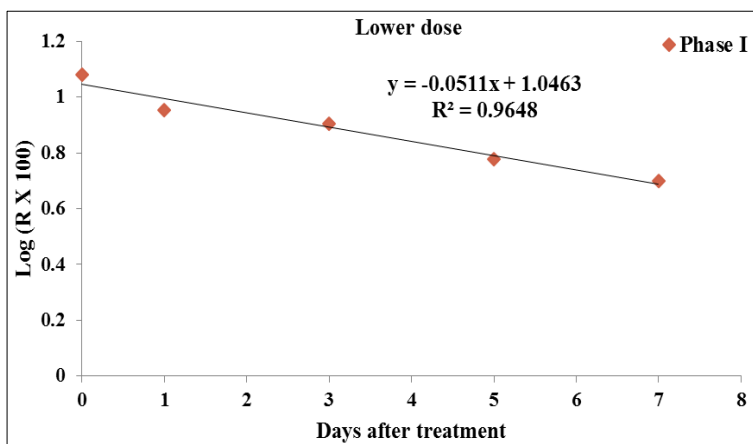


Fig 4: *In vitro* dissipation pattern of *S*-metolachlor at lower and higher dose in 9.2 pH water

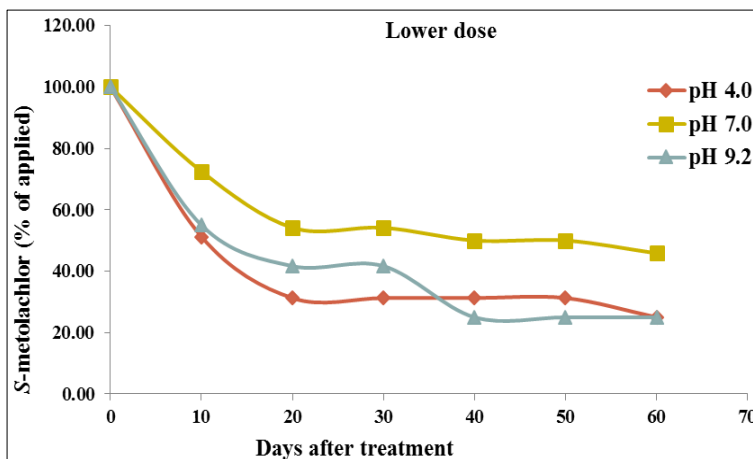


Fig 5: Degradation kinetics of *S*-metolachlor at three different pH water (4.0, 7.0 and 9.2) at lower dose

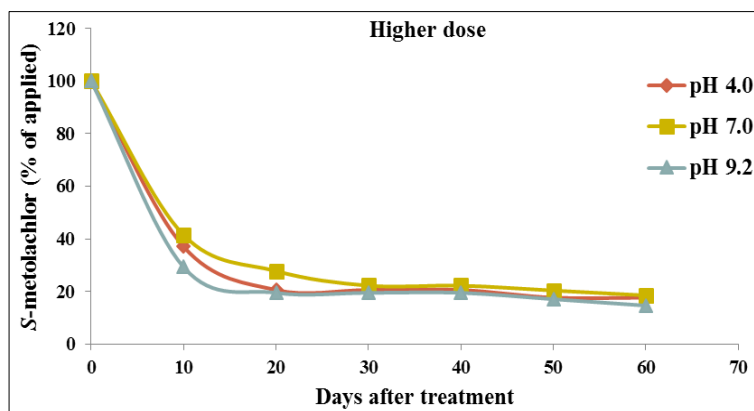


Fig 6: Degradation kinetics of *S*-metolachlor at three different pH water (4.0, 7.0 and 9.2) at higher dose

Conclusion

In vitro dissipation of *S*-metolachlor in water with pH 4.0, the rate was monophasic and biphasic at lower and higher dose, respectively. At lower dose the half-life value was 6.02 days. At higher dose the half-life values were 3.16 and 150.5 days during phase-I (0-7 days) and phase-II (7-60 days), respectively. In case of water with pH 7.0, the rate of *S*-metolachlor degradation was biphasic irrespective of the dose. At lower dose, the half-life values were 12.5 and 301 days during phase-I (0-10 days) and phase-II (10- 60 days), respectively. At higher dose, such values were 3.4 and 100.3 days during phase-I (0-10 days) and phase-II (10-60 days), respectively. For water samples with pH 9.2, the rate of dissipation was biphasic and triphasic in nature for lower and higher dose, respectively. At lower dose, the half-life values were 5.9 and 60.2 days during phase-I (0-7 days) and phase-II (7-60 days, respectively. At higher dose, the half-life values were 1.8, 12.04 and 150.5 days during phase-I (0-3 days), phase-II (3-10 days) and phase-III (10-60 days), respectively.

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