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Research Article

The Fate and Behaviour of Acetochlor 90% Emulsifiable Concentrate in Water Maintained at Different pH and Soils of Different Agro-Climatic Zones in India Under Laboratory Condition

Sayan Ghosh[1], Arijita Bhattacharyya*[,2], Sambrita Majumder[1], Bappa Ghosh[2], Sankhajit Roy[1], and Anjan Bhattacharyya[1]

[1]Department of Agricultural Chemicals, Bidhan Chandra KrishiViswavidyalaya, Mohanpur, Nadia, West Bengal 741 252, India
[2]Department of Chemistry, University of Kalyani, Kalyani, Nadia, West Bengal 741 235, India

Abstract

A laboratory study was performed to determine the residual fate of a herbicide formulation acetochlor 90 % EC in both aqueous system of different pH levels viz. 4.0, 7.0 and 9.2 and soil system collected from different agro climatic zones such as new alluvial soil, lateritic soil, coastal saline soil and black soil at recommended dose of 1 ppm (T_1) and double the recommended dose of 2 ppm (T_2) in a maintained dark area at 28 ± 2 °C temperature with 60 percent water holding capacity. Samples collected on 0 (2 h), 3, 7, 15, 30, 45, 60 and 90 days after treatments were processed for residue analysis of acetochlor. Extraction and clean up method was developed and the residue of acetochlor analyzed by gas chromatography equipped with electron capture detector (GC-ECD). The recovery % of acetochlor in every case was more than 85%. The half-life values of acetochlor were ranging from 5.4-23.1 days in water and 2.2-16.7 days in soil samples. Dissipation was approximated to first order kinetics in both conditions having correlation coefficient ranging from 0.969 to 0.997.Different pH and microbial activity with different organic carbon percentage contributed toward the degradation of acetochlor in soil.

Keywords: Acetochlor, residue, water, pH, soil, GC-ECD, half-life.

***Correspondence**
Author: Arijita Bhattacharyya
Email: arijitamam@gmail.com

Introduction

Acetochlor [2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide] is a selective pre-emergence chloroacitanilide herbicide first registered in USA in1994 for controlling annual grasses and broad leaf weeds in maize. Acetochlor is very much effective against those weed species which became resistance to several other herbicides such as 2,4-D, metolachlor, alachlor, EPTC, butylate, atrazine etc [1]. So, it can easily be expected that the use of acetochlor in crop field will increase day by day. Though crop protection is top priority for agricultural yield improvement but the widespread use of pesticide leads to environmental contamination in soil, ground water and surface water. Gavrilescu, 2010 and Miclean et al., 2011 [2, 3] reported that organochlorine compounds are toxic, bioaccumulative and tend to persist due to the lipophilic characteristics Water solubility of acetochlor is 233 mg/L. Behaviour and fate in soil is determined by different processes involving chemical degradation, microbial degradation sorption and binding by organic and mineral components, uptake by plant roots and volatilization. Persistence study in topsoil is one of the basic research in the assessment of the fate and behaviour of all chemical substances, including herbicide as a plant protection chemical. The half-life under field conditions should be less than laboratory study due to absent of different environmental factors [4]. Besides of various soil factor and different pH condition pesticide concentration plays an important role for its persistence behaviour and to maintain a safe practice for its use for human health concern.

Therefore, it is necessary to find out the residual fate of acetochlor in the environmental components such as soil, water and plant systems. Several scientists performed different kinds of research works to study the degradation behaviour of acetochlor under both field and laboratory condition [5-11]. Beside this, microbial degradation of acetochlor under laboratory condition also has been performed [12].

The main importance behind these types of research work is to monitor pesticide residue because the presence of pesticide residue in the environment for long time becomes hazardous towards human being and other living organisms and causes pollution. This type of observation becomes more essential when any new pesticide or new formulation is introduced in a new location. Acetochlor 90% EC is one type of new pesticide formulation introduced in India by M/s Sinochem India Co. Pvt. Ltd., New Delhi about which information regarding its residual fate is not available. In this respect, the following study has been undertaken to determine the residual fate and behaviour of acetochlor 90% EC formulation in water maintained at different pH and soils of different agro-climatic zones of India under the laboratory simulated condition to find out their probable effect on stability of acetochlor under different conditions. This experiment was performed at Pesticide Residue Laboratory, Department of Agricultural Chemicals, Bidhan Chandra KrishiViswavidyalaya, West Bengal, India.

Materials and Methods

Chemical reagents

Analytical grade standard of acetochlor (99.0%) was obtained from Sigma Aldrich Company. HPLC grade ethyl acetate procured from Rankem was used for this experiment. Other reagents which were used in the total experiment were of laboratory grade. Distilled water used for entire study was obtained from the laboratory distillation unit.

Preparation of water samples

Buffer capsules (Rankem) of pH 4.0, 7.0 and 9.2 were used for preparation of different buffer solutions. One capsule was used for 100 mL of distilled water to maintain the above mentioned pH. In a series of 250 mL conical flask 100 mL distilled water was taken and one capsule of different pH was added to each of the conical flask separately. The conical flasks were then left at room temperature for overnight for homogeneous mixing.

Collection and preparation of soil samples

In the present investigation, soils having various physico-chemical properties were considered for laboratory study of acetochlor 90% EC formulation in soil. All the gross soil samples were collected from different agro-climatic regions of India such as new alluvial soil from Mohanpur (22°59´N/88°29´E), lateritic soil from Jhargram (22°27´N/86°59´E), coastal saline soil from Canning (22°19´N/88°40´E) and black soil from Pune (18°32´N/73°52´E)from 0 to 15 cm depth with the help of a soil auger from ten number of spots in each case following 'zig-zag' technique of soil sampling. Then, are presentative portion of gross field sample was packed and transported to the laboratory for analysis. All the soils were air dried, ground and passed through a 0.2 mm sieve and sub-samples were taken by the usual methods of quartering to prepare laboratory sample. The study was performed at 60% of maximum water holding capacity of the soil and the moisture content of the soils was maintained with the addition of distilled water. The physico-chemical properties of the different soils (**Table 1**) were analyzed by different methods depicted as follows: soil texture was determined by the hydrometer method [13]. Soil pH was measured in soil + deionised water (1 + 2.5 by weight) [14]. The organic carbon content of the soil was determined by Walkley and Black wet oxidation method [15].

Table 1 Physico-chemical characteristics of different soils

Physico-chemical properties of soil	New alluvial soil	Lateritic soil	Coastal saline soil	Black soil
Soil order	Mollisol	Aridisol	Alfisol	Vertisol
Location	Mohanpur	Jhargram	Canning	Pune
pH	7.02	5.45	7.60	8.14
Organic Carbon (%)	1.00	0.64	1.03	0.67
Sand (%)	12	54	50	16
Slit (%)	65	16	23	24
Clay (%)	23	30	27	60

Laboratory study

For periodic incubation study, acetochlor was fortified to 100 mL water samples (of different pH) from the 100 ppm stock solution of the acetochlor 90% EC formulation in a manner to give a final concentration of 1 ppm and 2 ppm of active ingredient (a.i.) for single (T_1) and double (T_2) dose respectively. Similarly, to study the fate of acetochlor in different soils, 10 g of each type of soil sample was taken in 100 mL conical flask and fortified from same stock in a manner that final concentration of soil sample became 1 ppm and 2 ppm of a.i. for single and double dose respectively. One set of untreated control blank was simultaneously maintained in both of the cases of water and soil throughout the experimental period. Each treatment was replicated thrice in both of the cases of water and soil. The experimental flasks were placed in dark at B.O.D incubator and incubated at 28 ± 2 °C temperature. Incubator temperature was maintained by temperature controller. The samples were collected periodically for residue analysis of acetochlor at an interval of 0 (2 hour after application), 3, 7, 15, 30, 45, 60and 90 days.

Extraction of water samples

To analyze the acetochlor residue in water,100 mL water sample, after addition and thorough mixing with 10 g sodium chloride (Merck) was taken into a separatory funnel. The water sample was then partitioned with 100 mL ethyl acetate. The aqueous layer thus obtained was further partitioned with 50 mL of ethyl acetate twice. The organic phases of each time were combined after passing through activated sodium sulphate (SRL) and subsequently concentrated using rotary vacuum evaporator below 40ºC and volume was reconstituted with 10 mL ethyl acetate. Then the sample was filtered with the help of syringe filter through 0.22µm membrane filter paper and transferred into the vials and analyzed in gas chromatography equipped with electron capture detector (GC-ECD).

Extraction of soil samples

For analyzing residue of acetochlor in soil samples, 10g soil sample was placed in a 50 mL polypropylene centrifuge tube address with10 mL distilled water and subjected to vortex for 2 minutes. After that, 10 mL ethyl acetate, 4 g of activated sodium sulphate and 1 g of sodium chloride were added to the mixture and vortexed for 2 minutes. The sample was subjected to rotospin for 15 min @ 50 rotation per minute (r.p.m.) followed by centrifugation for 5 minutes at 5000 r.p.m speed. After centrifugation, an aliquot of 2 mL supernatant organic phase was collected with the help of micropipette to polypropylene centrifuge tube containing 25 mg of primary secondary amine (Varian), 25 mg activated sodium sulphate and 25 mg florisil (Acros Organics) and vortexed for 30 seconds and centrifuged at 6000 r.p.m. speed for 5 minutes. After that the clear extracts were filtered with the help of syringe filter through a 0.22 µm nylon filter paper into vials for GC-ECD analysis.

Instrumental parameters

Residues of acetochlor was determined on Agilent 6890N gas chromatograph equipped with electron capture detector and wide bore HP-5 column (30 m x 0.32 mm i.d. x 0.25 µm film thickness). For detection of acetochlor, the carrier gas (N_2) flow rate was maintained @ 0.5 mL/min (with a makeup flow @ 59.5 mL/min). The injector, oven and detector temperatures were maintained at 275°C, 210°C and 300°C respectively. Injection volume was maintained at 1µL in a split mode of 5:2ratio.The total run time was 10 minutes and retention time (R_T) of acetochlor was at 3.05 ± 0.1 min.

Calibration Curve Preparation

A calibration curve was prepared by plotting detector response to different concentrations of standard solution of acetochlor in the range of 0.01, 0.02, 0.05, 0.10, 0.50 and 1.00ppm.

Recovery Study

The recovery experiment was carried out on each type of untreated water and soil samples for validation of the analytical method and to know the efficiency of the extraction and clean up steps performed under the following experiment. The recovery experiment was conducted by fortifying acetochlor from 100 ppm stock prepared by its analytical standard into water of different pH (viz. 4.0, 7.0 and 9.2) and different kinds of soil (viz. new alluvial soil, lateritic soil, coastal saline soil and black soil) at 0.05 ppm, 0.1 ppm and 0.5 ppm levels.

Results

Linearity and estimation of calibration curve

High determination coefficient (R^2) of 0.99 was observed. The limit of detection (LOD) and limit of quantification (LOQ) were determined as 0.01 ppm and 0.05 ppm respectively.

Recovery for method efficiency

The average recovery percentages were ranging from 90.43-94.00 in case of water samples and 90.80-93.22 in case of soil samples (**Table 2** and **Table 3**) which complies with the SANTE guideline [16]. Method accuracy and precision was satisfactory in terms of mean recoveries in the range 70–110% associated with acceptable RSD (≤20%) respectively.

The dissipation of acetochlor 90% EC formulation in aqueous system at different pH levels and different kinds of soilsis presented in **Table 4** and **Table 5** respectively. No residue was detected in the control samples during the entire study. The residue gradually decreased with time following first order kinetics in all the cases.

Table 2 Recovery study of Acetochlor in soil

Substrate	Amount fortified (in µg/g)	Amount recovered* (in µg/g)	% Recovery	Average % Recovery
Mohanpur soil (pH 7.02)	0.050	0.047	94.62	92.61
	0.100	0.091	91.13	
	0.500	0.460	92.09	
Jhargram soil (pH 5.45)	0.050	0.045	89.53	93.22
	0.100	0.094	94.00	
	0.500	0.479	95.76	
Canning soil (pH 7.60)	0.050	0.045	90.40	92.00
	0.100	0.092	91.82	
	0.500	0.469	93.79	
Black soil (pH 8.14)	0.050	0.045	90.00	90.80
	0.100	0.092	91.58	
	0.500	0.455	91.08	

* Average of three replicates

Table 3 Recovery study of Acetochlor in water

Substrate	Amount fortified (in µg/mL)	Amount recovered* (in µg/mL)	% Recovery	Average % Recovery
pH 4.0	0.050	0.044	88.65	91.22
	0.100	0.093	93.00	
	0.500	0.460	92.00	
pH 7.0	0.050	0.044	89.29	90.43
	0.100	0.092	92.00	
	0.500	0.45	90.00	
pH 9.2	0.050	0.05	100.00	94.00
	0.100	0.09	90.00	
	0.500	0.46	92.00	

* Average of three replicates

Dissipation of acetochlor in different water system

The initial concentration of acetochlor in aqueous system was ranging from 0.86 ppm to 0.88 ppm in for single dose and 1.71 ppm to1.77 ppm for double dose. At pH 4.0 and pH 7.0 more than half of initial residue was dissipated 15 days after application for both of the doses, whereas at pH 9.2, more than 50% of initial residues dissipated within 7 days. The residue of acetochlor became below determination level at 90 days after application in case of single dose at pH 4.0 and pH 7.0 and in case of pH 9.2, single dose of acetochlor was not determined after 30 days of application. In case of double dose, the residue of acetochlor was still present in water system at pH 4.0 and pH 7.0 conditions after 90 days of application but in pH 9.2 condition, acetochlor residue was not found after 30 days of application. A calculated half-life value of acetochlor in water (Table 4) was lowest at alkaline pH (5.4-6.0 days) as compared to acidic pH (16.7-20.0 days) and neutral pH (18.8-23.1 days). According to Pesticide Property Data Base [17] acetochlor become stable at pH 5 and pH 7 condition and moderately stable at pH 9 condition which is to some extent similar to our study. The residue dissipation pattern of acetochlor in different water system is represented in figure 1.

Dissipation of acetochlor in different soil system

The initial concentration of acetochlor was found to vary from 0.86 ppm to 0.89 ppm and 1.74 ppm to1.76 ppm respectively for single dose and double dose respectively in soil systems. Both for single and double dose of acetochlor, more than half of the initial residue was dissipated in black soil 3 days after application and in case of other three types of soils, it was happened near about 15 days after application for both single and double dose. No residue of acetochlor was present in any soil sample after 90 days of application irrespective of doses. The calculated half-life value of acetochlor (Table 5) for new alluvial soil was found in the range of 15.8-16.7 days followed by lateritic soil where half-life values were 13.0-14.3 days. The half-life value was to some extent lower in coastal saline soil which was in the range of 8.1-10.3 days. Degradation rate was highest in black soil where half-life value was in the range of 2.2-4.1 days. According to U.S. Environmental Protection Agency, acetochlor is moderately persistent in soil. Some scientists (7) also observed that the half-life of acetochlor in medium silty loam soil (pH 6.4) was 12.4 days and in loamy sand soil (pH 5.5), the half-life value of acetochlor was 15.4 days. Figure 2 represent the decline pattern of acetochlor in different soil system under laboratory condition.

Table 4 Dissipation of acetochlor in different water

Dose	**Mean residue (in ppm) ± SD (% Dissipation)**								**Regression equation (determination coefficient: R^2)**	**Half life ($T_{1/2}$)**
	0 DAA	**3 DAA**	**7 DAA**	**15 DAA**	**30 DAA**	**45 DAA**	**60 DAA**	**90 DAA**		
Acidic water (pH 4.0)										
T_1	0.88± 0.04 (-)	0.79±0.03 (10.23)	0.59±0.03 (32.95)	0.42±0.03 (52.27)	0.29±0.03 (66.67)	0.17±0.02 (80.30)	0.08±0.01 (90.91)	BDL	y=-0.018x+ 2.920(0.994)	16.7 days
T_2	1.77± 0.08(-)	1.53±0.03 (13.56)	1.19±0.02 (32.77)	0.78±0.02 (55.93)	0.57±0.04 (67.98)	0.33±0.02 (81.17)	0.16±0.02 (90.96)	0.08±0.01 (95.48)	y= -0.015x+ 3.167(0.974)	20.0 days
Neutral water (pH 7.0)										
T_1	0.87± 0.03(-)	0.86±0.02 (12.98)	0.58±0.03 (33.59)	0.41±0.03 (52.67)	0.27±0.03 (68.70)	0.16±0.02 (81.30)	0.08±0.01 (90.46)	BDL	y = -0.016+ 2.909(0.992)	18.8 days
T_2	1.71± 0.04(-)	1.52±0.03 (11.09)	1.26±0.02 (26.65)	0.81±0.03 (52.72)	0.56±0.04 (67.32)	0.40±0.02 (76.85)	0.17±0.02 (90.08)	0.12±0.03 (93.19)	y = -0.013+ 3.179(0.969)	23.1 days
Alkaline water(pH 9.2)										
T_1	0.86± 0.03(-)	0.52±0.03 (39.77)	0.33±0.02 (61.78)	0.12±0.02 (85.71)	BDL	BDL	BDL	BDL	y = -0.055x+ 2.906(0.994)	5.4 days
T_2	1.74± 0.03(-)	1.04±0.08 (40.31)	0.65±0.03 (62.38)	0.29±0.03 (83.49)	BDL	BDL	BDL	BDL	y = -0.050+ 3.199(0.988)	6.0 Days

BDL= below determination limit (< 0.05ppm); DAA= days after application

Table 3 Dissipation of acetochlor in different soil

Dose	**Mean residue (in ppm) ± SD (% Dissipation)**								**Regression equation (determination coefficient: R^2)**	**Half life ($T_{1/2}$)**
	0 DAA	**3 DAA**	**7 DAA**	**15 DAA**	**30 DAA**	**45 DAA**	**60 DAA**	**90 DAA**		
New alluvial soil (pH 7.02)										
T_1	0.86±0.05 (-)	0.65±0.03 (24.71)	0.52±0.04 (40.16)	0.34±0.02 (60.61)	0.21±0.03 (75.67)	0.10±0.01 (88.95)	BDL	BDL	y = -0.019x+ 2.881(0.987)	15.8 days
T_2	1.74±0.06 (-)	1.28±0.05 (26.49)	0.98±0.03 (43.38)	0.69±0.04 (60.46)	0.41±0.03 (76.39)	0.24±0.02 (86.18)	0.11±0.02 (93.47)	BDL	y = -0.018x+ 3.165(0.988)	16.7 days
Lateritic soil (pH 5.45)										
T_1	0.89±0.04 (-)	0.79±0.05 (11.61)	0.59±0.04 (34.08)	0.38±0.03 (57.29)	0.16±0.02 (82.02)	0.08±0.02 (91.01)	BDL	BDL	y = -0.023x+ 2.944(0.997)	13.0 days
T_2	1.75±0.08 (-)	1.56±0.04 (11.22)	1.17±0.04 (33.46)	0.76±0.02 (56.65)	0.41±0.04 (76.43)	0.19±0.03 (89.35)	0.09±0.02 (94.49)	BDL	y = -0.021x+ 3.230(0.997)	14.3 days
Coastal saline soil (pH 7.60)										
T_1	0.89±0.03 (-)	0.65±0.03 (27.34)	0.46±0.02 (48.31)	0.21±0.03 (76.40)	0.07±0.01 (92.51)	BDL	BDL	BDL	y = -0.037x+ 2.925(0.996)	8.1 days
T_2	1.75±0.03 (-)	1.22±0.07 (30.34)	0.83±0.04 (52.67)	0.47±0.02 (73.28)	0.16±0.04 (90.84)	0.08±0.01 (95.42)	BDL	BDL	y= -0.029x+ 3.162(0.984)	10.3 days
Black soil (pH 8.14)										
T_1	0.88±0.04 (-)	0.31±0.02 (64.64)	0.10±0.01 (88.59)	BDL	BDL	BDL	BDL	BDL	y = -0.134+ 2.924(0.996)	2.2 days
T_2	1.76±0.06 (-)	0.83±0.06 (52.84)	0.45±0.03 (74.43)	0.13±0.02 (92.42)	BDL	BDL	BDL	BDL	y = -0.072+ 3.188(0.989)	4.1 days

BDL= below determination limit (< 0.05ppm); DAA =days After Application

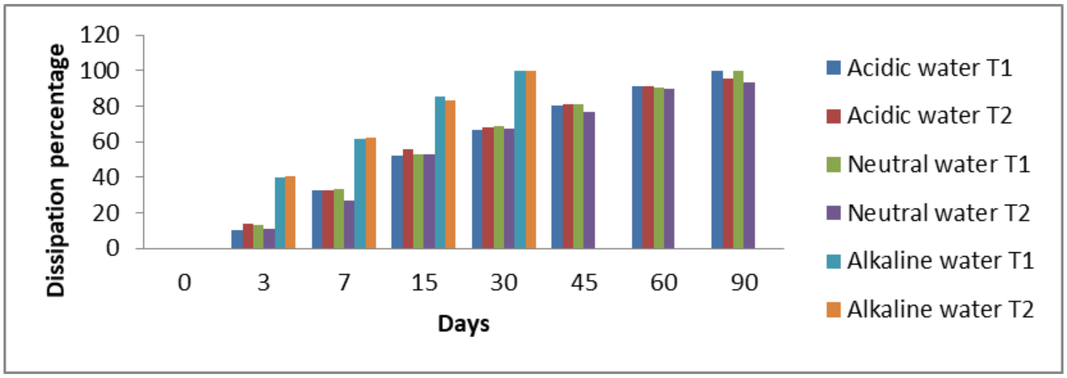

Figure 1 Residue dissipation graph of acetochlor in different PH water

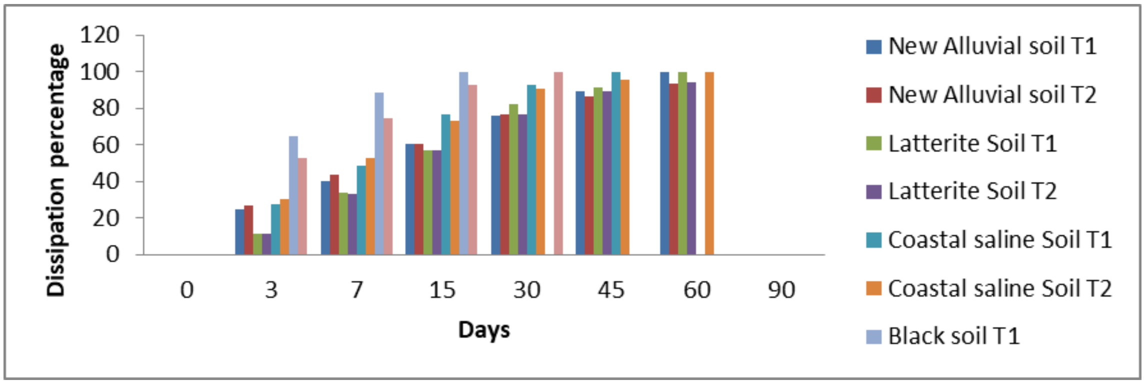

Figure 2 Residue dissipation graph of acetochlor in different Soil

Discussion

From the above results, it can be stated that the degradation rate of acetochlor was mostly affected by the pH of both water and soil. The rate of hydrolysis of acetochlor was pH dependent which was reflected by half-life value and supported by Carlson et al., 2006 [18] as reported earlier in his study that hydrolysis reaction occur in different acidic and basic condition for chloroacetanilide herbicide which also includes the N alkoxy methyl type herbicide acetochlor. In both of the cases of water and soil, degradation rate of acetochlor was faster in alkaline conditionand followed by acidic and neutral condition. External factors such as sunlight, temperature etc. have no such effect on the experiment as because it was performed under laboratory simulated condition. The relatively faster degradation of acetochlor in alkaline aquaous medium may be due to the hydrolysis reaction with water nucleophile to give hydroxy substituted and amide hydrolysis product [18]. As reported by Carlson et al [18], the hydrolysis reaction of N alkoxymethyl herbicide e.g. acetochlor in acidic medium occurred very slowly by N-dealkylation process to form primary amine and in neutralized condition it takes more time to convert. Beside pH, microbial populations also play a role in acetochlor degradation in every case under laboratory simulated condition because in our experiment the soil samples were not sterilized before application of pesticide. The experiment was performed in dark at 28 ± 2 °C temperature which was suitable for microbial growth. The microorganism population has ability to degrade a pesticide either by means of co metabolism or as source of food and energy. Soil metabolism of chloracetanilide herbicide was reported by Paul. C. Feng in 1991[19]. The major metabolites which were identified by their study were acidic in nature as they include oxanilic, sulfonic and sulfenyl acetic acid as the major site of transformation is the chlorine bearing carbon atom. Though organic C % is greater in coastal saline soil leading greater microbial population but the faster degradation of acetochlor in black soil than this may be explained by the alkaline pH effect. So, we can say that the main cause of acetochlor degradation was hydrolysis and by microbial population under laboratory simulated condition.

Conclusion

The present research work exhibits an effective and reliable method of extraction and analysis of acetochlor from different pH water and soil texture system with different pH. Acetochlor degrades more rapidly in alkaline medium with respect to neutral and acidic medium. Degradation study in four different soils showed good correlation between dissipation rate and content of organic matter in soil. Higher the organic matter content faster was the rate of dissipation. The outcomes of our study may help for assessing environmental safety due to application of acetochlor in agricultural field in India.

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