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Research Article

Risk Assessment, Decline Pattern and Residue Analysis of a Chloroacetanilide Herbicide Acetochlor in Rice Field System

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Abstract

Field experiments were conducted in a rice field as a test crop during two consecutive *kharif* seasons in India to study the dissipation kinetics and the harvest time residue of acetochlor in soil and rice plant and other co products. The formulation was applied at recommended (150 mL ha^{-1}) and its double dose (300 mL ha^{-1}) along with control which were replicated thrice in randomized block design. The soil and plant samples collected at periodical intervals for residue determination by Gas chromatography equipped with Electron Capture Detector (GC-ECD). Dissipation of acetochlor in both the system followed first order kinetics with mean half-life ranging from 9.41 - 11.36 days and 10.27 -11.90 days for rice plant and field soil respectively. No residue of acetochlor was detected in any sample at the time of harvest. This could not result as a threat to humans or the agricultural environment if it is applied in the crop field with proper recommendation.

Keywords: Acetochlor, dissipation, GC-ECD, half life, rice

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Introduction

Rice (*Oryza sativa* L.) is the preeminent principal food crop and chief grain in India. It plays a vital role in our food security. India is one of the largest producers of this basic food crop. Rice production thus contributes an important part of the national economy [1]. Rice is life for millions of rural households. It is also world's largest cereal crop which fulfils the caloric and nutritive need of million people. However, at the current rate of population growth, rice production has to enhance to about 140 million tons by 2025 [2]. Hence, a continuous research is going worldwide to increase the productivity of the rice crop. But the main problem in rice cultivation is due to weed infestation which is not managed timely by manually due to labour scarcity and high manual weeding cost. Thus, the use of herbicides as a chemical tool for weed control is supported in rice production. Herbicides are used routinely in rice field which results in migration of residues into environment and also in plant. Herbicides are applied in recommended dose may not pose serious problem for environmental pollution [3]. But, when used repeatedly with higher dosage some unintended negative impacts may occur which includes persistence in soil, pollution of ground water, toxic residues in food (contamination), feed and fodder and adverse effect on non-target organisms [4]. As a result rice grain quality may comprise by its residue also. Acetochlor, 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)-acetamide is a member of chloroacetanilide herbicide used in pre emergent condition mainly in maize and rice fields to control selectively annual grasses and broadleaf weeds [5-8]. But the literature study is few and limited under field condition, especially under West Bengal agro-climatic condition in India where 'rice is life slogan' is perfect. Acetochlor is absorbed by shoots and germinating plants roots which inhibit cell division by blocking protein synthesis [9]. Many studies reported the toxic, bioaccumulative and persistence nature of organochlorine pesticides due to their lipophilic characteristics [10, 11]. Such phenomenon constitutes a serious problem to cropping and herbicide residues in rice. Earlier studies reported that acetochlor has a risk of soil contamination [12, 13]. The pollution of environment compartment (mainly the soil as it is the first source of introduction of herbicide into the food chain) and different part of plant by acetochlor involves a serious risk to the environment and also to human health by direct exposure or through residues in food. So, the use of agricultural chemicals undoubtedly raises questions about the fate of the active substance. However, MRLs for rice crops have not yet been established in India for acetochlor. To ensure the safe use of acetochlor in the rice and to provide data for developing a MRL for rice which further helps in residue monitoring and quality control of food, it is important to determine the residues in rice crop fractions (rice grain, husk and straw), and soils and to study its dissipation kinetics.

In this study, considering the complex nature of rice plant matrix a simple and reliable analytical method of sample extraction using ethyl acetate [14] coupled with GC-ECD instrument was developed to evaluate acetochlor residues in rice system under field condition. The dissipation pattern of the herbicide in plant and soil, as well as the final residues in rice grain, husk and straw, were investigated. The results highlight the dissipation kinetics and residue of the acetochlor in rice cropping systems and contribute to risk assessment of acetochlor residues in rice which produce proper and safe application of acetochlor in rice fields under scientific guidance.

Materials and Methods

Field trials were conducted at experimental farm of Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, Nadia, West Bengal during two consecutive *kharif* (monsoon) seasons in a randomized block design with three replications of each dose and control. Plot size was 4 m x 5 m per replication per treatment. The climatic conditions during the trial period are presented in **Table 1**.

Table 1 Meteorological data

Season	Month	Average Temperature (°C)		Average Rainfall (mm)	Average Relative Humidity (%)	
		maximum	minimum		maximum	minimum
Season I	June	35.00	26.12	10.28	96.00	75.23
	July	32.66	25.32	8.04	95.19	82.29
	August	31.67	24.35	11.02	96.77	83.52
	September	32.47	24.51	8.01	95.63	80.87
	October	30.50	22.91	0.57	97.32	81.45
Season II	June	35.33	27.02	6.03	92.39	74.96
	July	32.57	26.20	8.43	95.93	84.81
	August	32.22	25.53	4.96	95.57	82.94
	September	32.21	25.01	8.46	96.73	82.03
	October	30.73	23.99	2.54	92.52	62.33

The herbicide formulation acetochlor 90% EC was applied for one time @ 150 mL ha^{-1}(recommended dose or T_1) and 300 mL ha^{-1}(double the recommended dose or T_2) at a spray volume of 500 Lha^{-1} with Knapsack sprayer in field soil after three days of transplanting of rice seedlings. Green rice leaves and soil were collected from each replicated plot at 0 (2 h after application), 3, 7, 15, 30, 45 and 60 days after application. Grain, husk and straw were collected at harvest. Soil sample was also collected at the time of harvest using Z method. Sub samples of 250 g of rice green leaves, harvested straw, grain samples and 500 g soil sample were brought to the laboratory immediately after collection. Grain, husk were collected from harvested rice plant and remaining portion used as straw and extracted for residue analysis. The Physico-chemical properties of new alluvial soil samples collected from West Bengal, Mohanpur (22°59´N/88°29´E), an agro-climatic region of India (**Table 2**) were analyzed by different methods depicted as follows: soil texture was determined by the hydrometer method [15]. Soil pH was measured in soil + deionised water (1 + 2.5 by weight) [16]. The organic carbon content of the soil was determined by Walkley and Black wet oxidation method [17]. Samples were collected from 0 to 15 cm depth with the help of a soil auger from ten numbers of spots in each case following 'zig-zag' technique of soil sampling. Then, a representative 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.

Table 2 Physicochemical property of soil

Physico-chemical properties of soil	New alluvial soil
Location	Mohanpur
pH	7.02
Organic Carbon (%)	1.0
Sand (%)	12
Slit (%)	65
Clay (%)	23

Analytical grade acetochlor (99.0%) was provided by 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 was obtained from the laboratory distillation unit. The stock solution of analytical standard of acetochlor was prepared by weighing 10 mg (± 0.01) of analyte in volumetric flask (certified class A-100mL) and dissolving in 100 mL ethyl acetate. From the stock solution the calibration standards were prepared by serial dilution using ethyl acetate (0.01 – 1.0 mg/kg).

Representative five gram of blended rice plant sample [rice green leaf/husk/ grain/ straw] was placed in a 50 mL centrifuge tube and 10 mL distilled water was added with it and vortex well for one minute. After that, 10 mL ethyl acetate followed by 4 g of activated sodium sulphate (SRL) and 1 g sodium chloride (Merck) were added in the tube containing sample. Then the sample vortex for 2 minutes and rotospin (Tarson) for 15 minutes @ 50 rpm speed. The sample was then centrifuged (Eltek) at 5,000 rpm speed for 5 min. After centrifugation, 4 mL of supernatant was collected and subsequently evaporated in the nitrogen evaporator (Turbo Vap, Caliper Life Science, USA). After that the volume was reconstituted with 2 mL ethyl acetate, taken in centrifuge tube containing 25 mg PSA (Varian), 25 mg sodium sulphate and 15 mg GCB (United Chemical Technology) and vortex for 30 seconds and then centrifuged at 6,000 rpm speed. for 5 minutes**.** After that the clear extracts were filtered with the help of 0.22 µ nylon filter paper and kept into the vials and analyzed in gas chromatograph (GC) equipped with Electron Capture Detector (ECD).

To analyze the residue of acetochlor in field soil, ten gram of representative soil sample was taken into 50 mL centrifuge tube and 10 mL water was added with it and vortex well for one minute. After that, 10 mL ethyl acetate followed by 4 g of activated sodium sulphate and 1 g sodium chloride were added in the tube containing sample. Then the sample vortex for 2 minutes and rotospin for 15 minutes @ 50 rpm speed. The sample was then centrifuged at 5,000 rpm speed for 5 min. After extraction, 2 mL soil extract was collected and cleaned up with 25 mg PSA, 25 mg florisil (Acros Organics) and 25 mg Na_2SO_4. Finally the sample was analyzed in GC-ECD.

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 temperature was set at 275°C, 210°C and 300°C respectively for detection of acetochlor. Injection volume was maintained at 1µL in a split mode of 5:2.The total run time was 10 minutes and retention time (R_t) of acetochlor under this condition of instrument was at 3.05 ± 0.2 min.

Results

In this study a calibration curve was prepared by plotting the areas of corresponding to different concentrations of standard solution of acetochlor viz. 0.01, 0.02, 0.05, 0.10, 0.50 and 1.00 mg/kg i.e six level of standards as well as in plant and soil matrix (matrix matched calibration curve) with a correlation coefficient (R^2) value of greater than 0.98. From this study the method LOD, based on signal-to-noise ratio of 3:1, was calculated by injecting matrix-matched standard mixture at the lowest calibration concentration. The LOQ was set at the lowest fortified concentration level of the analytes giving a response that could be quantified with satisfactory relative standard deviations (RSDs).The limit of detection (LOD) and limit of quantification (LOQ) were determined as 0.01 mg/kg and 0.02 mg/kg respectively.

Table 3 Recovery of acetochlor in different Substrate

Substrate	Fortified concentration (mg/kg)	Average amount recovered (mg/kg) (n=3)	Average recovery percentage ± RSD
Rice plant	0.02	0.0173 ± 5.78	86.67
	0.10	0.0907 ± 3.02	90.73
	0.20	0.1847 ± 5.42	92.33
Rice grain	0.02	0.0175 ± 7.70	87.67
	0.10	0.0911 ± 3.10	91.13
	0.20	0.1853 ± 4.87	92.67
Straw	0.02	0.0182 ± 4.95	91.00
	0.10	0.0923 ± 2.64	92.27
	0.20	0.1870 ± 1.07	93.50
Husk	0.02	0.0178 ± 6.55	89.17
	0.10	0.0920 ± 4.21	92.00
	0.20	0.1863 ± 4.56	93.17
Soil	0.02	0.0176 ± 6.84	88.17
	0.10	0.0911 ± 3.10	91.13
	0.20	0.1853 ± 4.87	92.67

*n= number of replication

The recovery of acetochlor from rice, its co products and soil was determined (**Table 3**) by analyzing fortified blank samples. Analysis was carried out at three concentration levels (0.02, 0.10 and 0.20 mg/kg) in three replicates. The recovery experiment was conducted by fortifying acetochlor from 100 mg/kg stock prepared by its analytical standard into rice plant and field soil samples. The average recovery for all concentration was above 90% for all the cases. This complies with the SANCO guidelines [18], which requires mean recoveries within the range 70–110% with RSD value less than or equal to 10%. So the extraction and cleanup methods became suitable for residue analysis.

The initial deposits of acetochlor in rice plant after two hours of spray were found to be 0.341 and 0.718 mg/kg corresponding to the T_1 and T_2 respectively in season I; which was dissipated nearly 25 percent or more and reached the value of 0.257 and 0.544 mg/kg (for T_1 and T_2 respectively) after 3 days. The half-life values of acetochlor in this season in rice plant sample were determined as 9.41 and 11.36 days for T_1 and T_2 respectively. For season II initial deposits of acetochlor in rice plant after two hours of spray were found to be 0.301 and 0.712 mg/kg corresponding to the T_1 and T_2 respectively which was dissipated to 0.223 and 0.510 mg/kg (for T_1 and T_2 respectively) after 3 days. The half-life values of acetochlor for this season in rice plant sample were determined as 8.73 and 10.83 days for T_1 and T_2 respectively. It was observed that for both the season residue decline pattern is same i.e at 15 days (**Table 4**) the residue of T_1 reached below quantification limit [BLOQ < 0.02 mg/kg] and in case of T_2, the residue of acetochlor become BLOQ at 30 days. No residue was detected in any control samples of rice plant.

Table 4 Residue of acetochlor presents in rice plant and field soil and at different time intervals after the pre-emergence application of formulation

Substrate	Season	Doses	Residue in mg/kg ± RSD (% dissipation) Days after Treatment (DAT) (n=3)						Regression equation	Half-life (Days)
			0	3	7	15	30	45		
Rice Plant	I	T_1	0.341±3.38 (-)	0.257±6.04 (24.56)	0.202±6.04 (40.61)	BLOQ	BLOQ	BLOQ	y = -0.032x + 2.5227 R^2 = 0.9833	9.41
		T_2	0.718±1.81 (-)	0.544 ±3.34 (24.28)	0.427 ±2.23 (40.53)	0.280±7.13 (61.00)	BLOQ	BLOQ	y = -0.0265x + 2.8327 R^2 = 0.9857	11.36
	II	T_1	0.301±5.30 (-)	0.223 ±5.86 (25.94)	0.171 ±6.56 (43.02)	BLOQ	BLOQ	BLOQ	y = -0.0345x + 2.4683 R^2 = 0.9853	8.73
		T_2	0.712±1.31 (-)	0.510 ±3.55 (28.34)	0.381±2.89 (46.46)	0.262±4.20 (63.14)	BLOQ	BLOQ	y = -0.0278x + 2.8138 R^2 = 0.9613	10.83
Field Soil	I	T_1	0.362±3.41 (-)	0.316±5.23 (12.88)	0.227±8.67 (37.35)	0.135±8.67 (62.83)	BLOQ	BLOQ	y = -0.0293x + 2.5693 R^2 = 0.9955	10.27
		T_2	0.591±2.29 (-)	0.518±2.71 (12.36)	0.371±5.14 (37.25)	0.221±8.48 (62.58)	0.106± 6.16 (82.11)	BLOQ	y = -0.0253x + 2.7632 R^2 = 0.992	11.90
	II	T_1	0.339±4.75 (-)	0.298±4.19 (12.00)	0.212±3.56 (37.46)	0.123±5.33 (63.72)	BLOQ	BLOQ	y = -0.0302x + 2.5439 R^2 = 0.9941	9.97
		T_2	0.576±1.77 (-)	0.492±2.75 (14.63)	0.385±4.02 (33.14)	0.257±4.89 (55.35)	0.095± 8.21 (83.57)	BLOQ	y = -0.026x + 2.7716 R^2 = 0.9969	11.58

*BLOQ = Below Limit of Quantification; *n= number of replication

The initial deposits of acetochlor in field soil after two hours of spray were found to be 0.362 and 0.591 mg/kg corresponding to the T_1 and T_2 respectively in season I; which was dissipated to 0.316 and 0.518mg/kg (for T_1 and T_2 respectively) after 3 days. The half-life values of acetochlor in this season in field soil sample were determined as 10.27 and 11.90 days for T_1 and T_2 respectively. For season II initial deposits of acetochlor in field soil after two hours of spray were found to be 0.339 and 0.576 mg/kg corresponding to the T_1 and T_2 respectively; which was dissipated to 0.298 and 0.492 mg/kg (for T_1 and T_2 respectively) after 3 days. The half-life values of acetochlor for this season in field soil sample were determined as 9.97 and 11.58 days for T_1 and T_2 respectively. It was observed that for both the season residue decline pattern is same i.e at more than 50 % of acetochlor was dissipated at 15 days (Table 4). The residue of T_1 reached below quantification limit (BLOQ < 0.02 mg/kg) at 30 days and in case of T_2, the residue of acetochlor become BLOQ at 45 days. No residue was detected in the control samples of both seasonal field cropped soil.

Discussion

The recent study for soil half life of acetochlor are similar as earlier reported by different scientists [19-22] and ranged from 2 to 18 days in field experiments. In the surface soil acetochlor has a great tendency to undergo highly chemical and biological loss and volatilization [23]. Acetochlor translocates in plant from soil by roots. The dissipation study and half life of rice plant firstly reported in this paper. There must be different environmental factor besides the physicochemical nature of the compound e.g temperature, light, rainfall, humidity for faster dissipation of herbicide. The explanation of obtained half life may be also supported by the soil study. As when herbicides are applied to soil, undergoes decomposition and some portion may be taken by plants accumulating in the edible parts. Variation in result with two different seasons may be attributed by the effect of the different climatic condition. The residue declines progressively with time taking residues at 0 days as initial deposit. In all the cases a straight line was found when the log of residue was plotted against time and thereby establishing that first order reaction kinetics was involved in the dissipation process in rice plant and field soil. It was found that the residues gradually decreased with time following 1^{st} order kinetics.

In the context of this study, the results indicated that 50% of the initial residues of the herbicides are dissipated in soil within 15 days and 7 days in plant after treatment. No residue of acetochlor was detected in any rice plant sample (grain, husk and straw) and field soil collected at the time of harvest.

The acceptable daily intake (ADI) for acetochlor 0.0036 mg/kgbw/day [24]. In this study, the final residues of the herbcide in the rice samples were all below the LOQs at harvest time. The LOQ acetochlor was 0.02 mg/kg. Therefore, the supervised trial median residue (STMR) value may be assumed to be at the LOQ. The national estimated daily intake (NEDI) of the herbicides was defined by the following equation: NEDI = STMR × Fi/bw [5] where the average body weight (bw) of an adult in India was estimated at 56 kg [25] and the intake of an adult per day (Fi) was 0.4 kg per indian person when consuming rice, which was provided based on the dietary guidelines issued by the National Institute of Nutrition India. According to the equation, the NEDI for acetochlor is 0.000142 mg/kg is fairly low; the daily dietary intake of acetochlor is 3.9 % of the ADI in India, Such results imply that the potential health risks induced by the two herbicides in this mixture are not significant in rice fields, even at double the recommended dosage.

Conclusion

The outcomes from this research experiment involving acetochlor residues in rice plant and field soil shows that the use of herbicide acetochlor following double the manufacturers' recommended dosage on rice are safe under an open field environment and could not result as a threat to humans or the agricultural environment if it is applied in the crop field with proper recommendation with government regulations. So if it is used properly also the food quality will be maintained.

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