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Degradation dynamics of clomazone in paddy field

A. BHATTACHARYYA, [1]B. GHOSH, S. P. DAS AND [1]A. BHATTACHARYYA

Dept. of Chemistry, University of Kalyani, Kalyani-741235, West Bengal

[1] Dept of Ag. Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur-741252,West Bengal

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ABSTRACT

To find out the persistence feature of Clomazone (50 EC), recently introduced in India was applied in paddy field, 3-5 days after transplantation of paddy. The study was conducted at BCKV farm, Mohanpur, Nadia during August to November, 2012. Clomazone 50 % EC was applied on paddy @ 1000 ml/ha and 2000 ml/ha along with untreated control. Cropped soil and plant samples were extracted separately with ethyl acetate: acetone (8:2 v/v) and subsequently the extract were cleaned up by using PSA, C_{18} & GCB. Then the extract was filtered and analysed in GC-MS/MS. The recovery percentage was ranged between 86-95% with LOQ 20 ngg^{-1} for all matrices. Following first order kinetics, the half-life of clomazone was found in ranges 0.91-1.13 days for cropped soil and 2.04 days for paddy plant respectively. From this study it was proved beyond doubt that Clomazone may not create any residual toxicity problem.

***Keywords*:** Clomazone, GC-MS/MS, grain, herbicide, paddy, residue, soil

Rice is one of the most important cereal food crops in term of both area, production and consumer preference in India (*Rice%20 profile.pdf* accessed on 24.7.2014) and supplier of 20% of worlds dietary energy (*nutrition@fao.org.*,2004). India, predominantly being an agricultural country is the second largest producer and consumer of rice and a major exporter of it (*FAOSTAT 2012*). Rice is the grain that has shaped the culture, diet and economics of billions of people in the world (*Ghosh et al*.,2013). Clomazone (2-(2-chlorobenzyl)-4,4-dimethyl-1,2oxazolid-in-3-one) is an isoxazolidinone group of broad spectrum, pre-emergence selective herbicide with both contact and residual activity which controls annual broad leaf weeds and grasses (*Prabha et.al*.,2006 and *EPA, Registration Review,2007*). It is a systemic herbicide that is taken up by plant roots and shoots and moves into the xylem, inhibiting the formation of photosynthetic pigments. This results in bleaching or whitening of plants. Clomazone toxicity is believed to be caused by a plant metabolite, 5-ketoclomazone, and is dependent upon a plant's ability to oxidize the parent compound to this active metabolite. Pest free and residue free agricultural products are becoming increasingly important (*Visalakhmi et al*., 2013). Clomazone is the only member of the isoxazolidinone family of herbicides currently in use (*EFSA Journal 2011*).The degradation pattern of Clomazone depends on environmental factors, particularly by moisture content, hence the degradation process is slower in soils with low organic matter content and lower precipitation and temperature (*Jelena et al*.,2012).The present paper deals with the persistence feature of clomazone in soil and plant after application of Clomazone 50% EC in the paddy field.

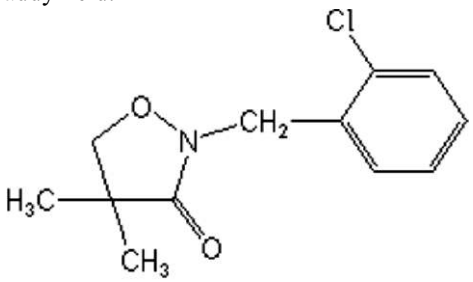

Fig. 1. Chemical structure of clomazone

MATERIALS AND METHODS

To determine the degradation dynamic of Clomazone in rice paddy plant and in field soil along with the residue study of rice grain, husk and straw at harvest time a field study was conducted at Jaguli Instructional Farm, BCKV, Mohanpur Nadia during August to November, 2012. All samples were collected at harvest time and subsequently analyzed Clomazone residue at laboratory. The herbicide formulation Clomazone 50% EC was applied in the above said field under West Bengal agro-climatic condition with the help of Knapsack sprayer @ 500 g a.i./ha (T_1) and @ 1000 g a.i./ha(T_2) in Randomized Block Designed(RBD) plots and maintained untreated control (T_3) plots. The area of each plot was $20m^2$ (12rows/plot). Three replications were used for each treatment.

For persistence behaviour study, paddy plant and field soil were collected at 0 (2 hr. after spraying), 1, 3,5,7,10,15 and 30 days after application of the herbicide. Paddy green foliage (0.5 kg) samples and

Email: arijitamam@gmail.com

field soil (1.0 kg) were collected from 5-7 places randomly in each treatment plots replication wise on each date of sampling and Paddy straw (0.5 kg), grain (0.5 kg), soil (1 kg) samples were taken at the time of harvest . Samples from untreated control plots were also collected in the same way. Soil samples were collected from a depth of 15 cm with the help of soil auger. Paddy plant samples were chopped, soil samples were air dried and grains were grinded to facilitate extraction of the herbicide using organic solvent. A valid representative (50 g) of plant (50 g each for green foliage, grain and straw) and soil samples for each compound were prepared separately by quartering technique in the laboratory and taken for quantification of herbicides residue analysis separately. The plant samples were homogenized using Polytron homogenizer (Model: Polytron, PT-MR-3100 Kinemetica AG, Lucerne, Switzerland) and extracted immediately after collection and extracts were cleaned up immediately after extraction.

The stock solution of analytical standard of Clomazone 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 (0.05-1ppm).

The Paddy samples (Paddy Straw/Husk/Paddy Grain) were grinded using a blender. Then five grams (5 g) of the sample was taken in a 50ml fluorinated ethylene propylene (FEP) centrifuge tube. To it 10 ml Ethyl Acetate: Acetone (8:2) was added and subjected to vortex (Spinix) for 2 mins and placed in rotospin (Tarsons) for 20 minutes. Then the sample was centrifuged using high-speed, refrigerated centrifuge, Model Avanti J-301(Beckman Coulter, Fullerton, CA) for 10 mins at 5000 rpm and 4 ml of supernatant liquid was taken in 15 ml centrifuge tube. Afterwards 50 mg C_{18}, 50 mg PSA, 50 mg GCB and 300mg Na_2SO_4 was added to it and vortex for 2 min. The sample was again centrifuged for 5 min at 10000 rpm. After centrifugation 3 ml supernatant was collected by micropipette and evaporated to dryness under Nitrogen evaporator and reconstituted in 1.5 ml ethyl acetate. Then the ethyl acetate extract filtered through 0.2µm nylon membrane filter and finally ethyl acetate extract was analyzed by GC-MS to quantify Clomazone content present on paddy samples.

Ten gram (10 g) soil was taken in a 50 ml (FEP) centrifuge tube. To it 10 ml Ethyl Acetate: Acetone (8:2) was added and subjected to vortex for 2 mins and placed in rotospin (Tarsons) for 20 mins.. Then the sample was centrifuged using high-speed, refrigerated centrifuge, Model Avanti J-301(Beckman Coulter, Fullerton, CA) for 10 mins at 5000 rpm and 4 ml of supernatant liquid was taken in 15 ml centrifuge tube. Afterwards 50 mg C_{18}, 50 mg PSA, and 300mg Na_2SO_4 was added to it and vortex for 2 min. The sample was again centrifuged for 5 min at 10000 rpm. After centrifugation 3 ml supernatant was collected by micropipette and evaporated to dryness under Nitrogen evaporator and reconstituted in 3 ml ethyl acetate. Then the ethyl acetate extract filtered through 0.2µm nylon membrane filter and finally ethyl acetate extract was analyzed by GC-MS to quantify Clomazone content present on paddy field soil samples.

The samples were analyzed with a Varian (Walnat Crrek, CA) Saturn 2200 mass spectrometer coupled to a model 3800 gas chromatograph. The mass spectrometer was used in the full scan mode with electron impact ionization. The system was equipped with a Model 1079 programmed temperature vaporizer injector, electronic flow control and autosampler (CTC COMBIPAL). The injection liner (single gooseneck 3.4 mm id) contained a plug of carbofrit (Restec Bollefonte, PA) to allow 9 µL injections of the ethyl acetate extracts. Varian MS Workstation Software (Version 5.1.2600.2180) was used for instrument control and data analysis.

GC Parameters:

Column : VF-5 MS, 30 m length (0.25 mm id, 0.25 µm film thickness)

Flow rate : 1.0 ml/ min

Run time : 16.50 min

Injection volume : 9 µl

Retention time : 10.69 ± 0.2 min

Column oven temperature programmed:

Temp (ºC)	Rate (ºC min^{-1})	Hold (min)	Total time (min.)
70	-	2	2
180	20	0	7.50
200	10	1	10.50
280	20	2	16.50

A linearity check study was carried out with the help of analytical standard. Also, to know the interference of each substrate, matrix matched

calibration standard for each substrate was prepared. In this study calibration curve was prepared by taking the areas corresponding to different concentrations of matrix match calibration standard, against which final quantification was done.

RESULTS AND DISCUSSION

Good linearity was observed in the studied range with R^2 e" 0.99, limit of detection (LOD) and limit of quantification (LOQ) considered when signal to noise ratio of 3:1 and 10:1. For clomazone Limit of detection was 0.005 ppm and Limit of quantification was 0.02 ppm for all the substrate. Recovery studies were carried out in order to establish the reliability of the analytical method and to know the efficiency of extraction and clean up steps employed for the present study. For recovery experiment, Clomazone was fortified to paddy straw, husk, grain and cropped soil. The results of the recovery experiment of Clomazone 50 EC in Paddy samples are presented in table 1.

Table 1: Recovery of clomazone in paddy straw, grain, husk and cropped soil

Substrate	Amount fortified (ppm)	Amount recovered* (ppm)	% recovery	Average % recovery
Straw	0.100	0.089	89.00	87.66
	0.050	0.042	84.00	
	0.020	0.018	90.00	
Grain	0.100	0.085	85.00	85.33
	0.050	0.043	86.00	
	0.020	0.017	85.00	
Husk	0.100	0.087	87.00	88.33
	0.050	0.044	88.00	
	0.020	0.018	90.00	
Cropped soil	0.100	0.088	88.00	86.33
	0.050	0.043	86.00	
	0.020	0.017	85.00	
Paddy plant	0.100	0.088	88.00	85.66
	0.050	0.042	84.00	
	0.020	0.017	85.00	

**Average of three replicates*

Table 2: Residue of clomazone presents in paddy plant and field soil at different times intervals after the application of formulation containing Clomazone 50% EC

	Clomazone 50% EC @ 500 g a.i.ha^{-1}				Clomazone 50% EC @ 1000 g a.i ha^{-1}			
Day after	Paddy Plant		Field Soil		Paddy plant		Field Soil	
application	*Meanmg kg^{-1} (RSD)	Dissipation (%)	*Mean mg kg^{-1} (RSD)	Dissipation (%)	*Mean mg kg^{-1} (RSD)	Dissipation (%)	*Mean mg kg^{-1} (RSD)	Dissipation (%)
0	0.08(8.4)	-	0.27(1.3)	-	0.09(6.17)	-	0.48(1.2)	-
1	0.05(12.12)	38.00	0.11(4.5)	60.00	0.07(10.09)	27.00	0.30(5.2)	38.00
3	BDL	-	0.03(16.80)	90.00	0.03(12.50)	64.00	0.05(13.0)	90.00
7	BDL	-	BDL	-	BDL	-	0.03(15.7)	94.00
10	BDL	-	BDL	-	BDL	-	BDL	-
Regression equation	-		y =-0.330 x + 2.408 R^2=0.994		y =-0.147 x + 1.954 R^2=0.999		y = -0.266x + 2.670 R^2=0.961	
Half-life ($T_{1/2}$)	-		0.91 Days		2.04 Days		1.13 Days	

*BDL: Below determination limit (d" LOQ: 0.02 mg kg-1) * Average RSD: Relative standard deviation*

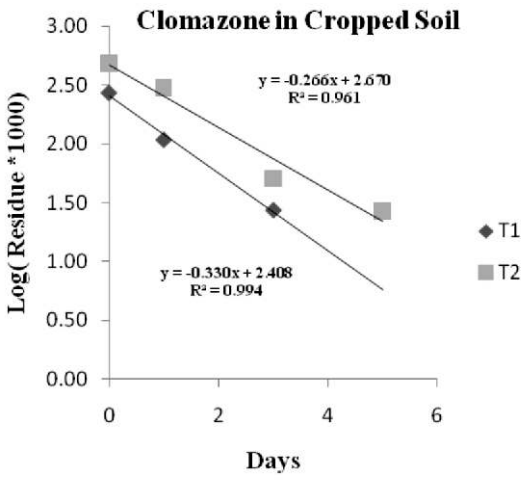

Fig. 2: Kinetic Curve of Clomazone in cropped soil (T_1(@ 500 g a.i.ha^{-1} and T_2@ 1000 g a.i.ha^{-1})

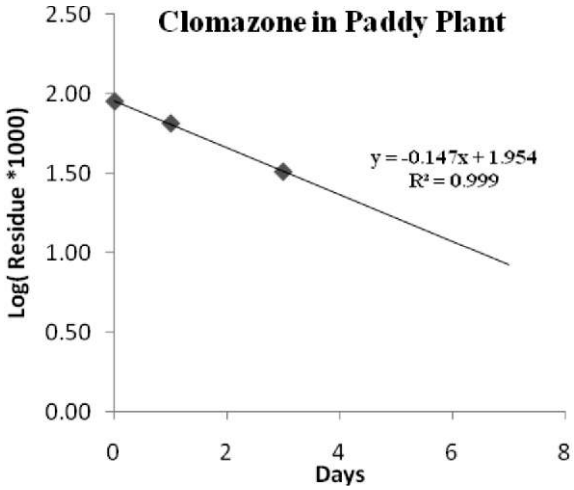

Fig.3: Kinetic curve of clomazone in paddy plant (T_2@1000 g a.i.ha^{-1})

It was observed from the table that the mean percent recovery for Clomazone at three fortification levels for all Paddy samples (straw, grain, husk and soil) was over 85%. As the recovery percentage is more than 85%, hence the method can be adopted for residue and dissipation study of Clomazone in Paddy sample. The average recovery of Clomazone was found to be 87.66%, 85.33%, 88.33%, 86.33% and 85.66% for straw, grain, husk and soil and paddy plant respectively. The present recovery data corroborates with the earlier findings as mentioned by *Dors et al.,* 2011, *Attallah et al.,* 2012 and *Antonius*, 2010.

The results of the persistence behavior of the herbicide formulation containing Clomazone in rice paddy plant and field soil have been summarized in Table 2. At the harvest time (120 days after application), residue of clomazone was below detected limit (d" LOQ: 0.02 ppm) in all samples (paddy straw, rice grain and field soil) for the season.

It was found that the residues of Clomazone in paddy plant and soil, gradually decreases with time. The rate was determined which follows 1st order kinetics. The initial deposits (2 h after spraying) of Clomazone in field soil were found 0.27 mgkg^{-1} (T_1) and 0.48 mgkg^{-1} (T_2) for the season and 0.08 mgkg^{-1} (T_1) and 0.09 mgkg^{-1} (T_2) for plant sample respectively. More than 50 % of the initial deposit was degraded within 3 days irrespective of this season in soil. The persistence of any chemical is generally expressed in terms of half-life ($T_{1/2}$) or DT_{50} i.e. time for disappearance of pesticide to 50 per cent of its initial concentration. $T_{1/2}$ values are often obtained by fitting first-order kinetics to observed degradation patterns as $C_t = C_o$ x exp(-kt) where Ct is chemical concentration (mg kg^{-1}), and k is the first order rate constant (h^{-1}) independent of Ct and C_0 (*Hoskin et al*., 1961). The $T_{1/2}$ of Clomazone was calculated using Hoskins formula. The dissipation pattern followed 1storder kinetics, the half-life of Clomazone was found in the range of 0.91-1.13 days for field soil and 2.04 days on paddy plant and at harvested crop produce that is rice grain, husk and paddy straw, soil samples Clomazone residue were not detected. As there is no Indian MRL of Clomazone in rice grain, the MRL of the compound is considered as reported in UK/EC *(UK/ECMRLs)* .So considering the above fact it may stated that rice grain is safe to consume. From this study it was proved beyond doubt that Clomazone may not create any residual toxicity problem.

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