



Persistence and Residue of Clomazone in Soil and Soybean by HPLC-DAD

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Recent concerns of ground and surface water contamination by some of the herbicides has lead to renewed interest on persistence and degradation behaviour of herbicides in soil environment. Thus, this study was conducted to assess the persistence and residue of clomazone in soil and soybean. The clomazone residue was extracted using methanol- water and determined by Agilent HPLC-DAD. The clomazone recovery from different matrices varied from 84 to 103 %, at fortification levels of 1 to 0.1 $\mu\text{g g}^{-1}$. More than 80 % of the applied clomazone degraded from soil within 14 days with mean half life of 4.9 days. Clomazone residues were found above the detection limit in soil and soybean only when it is applied at higher doses (1250 and 2000 g a.i. ha^{-1}) No clomazone residues ($< 0.01 \text{ mg/kg}$) were detected in any of the matrices at the lower concentrations of 750 and 1000 g a.i. ha^{-1} treatments. On the basis of clomazone persistence in soil and grain, a safe pre-harvest interval of 95 days for soybean after clomazone application is suggested.

Keywords: Clomazone, Persistence, HPLC analysis, Soybean.

INTRODUCTION

The agricultural soil is the final destination of a large number of herbicides, either when they are applied directly to the soil or on the shoots of plants [1]. When the herbicides, reach the ground, they interact with the environment and undergoes physical, chemical and biological degradation [2]. Recent concerns of ground and surface water contamination by some of the herbicides has led to renewed interest on dissipation behaviour of herbicides in soil and environment. Several monitoring programmes have also been implemented by different countries to check the environmental contamination and for ecological risk assessment by pesticides.

Clomazone is [2-(2-chlorobenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one] belongs to an isoxazolidinone herbicide produced by the FMC crops and was first approved for use in 1986 [3] within the United States. It is a selective herbicide used for the pre-emergence control of a variety of grasses and broad-leaved weeds. Clomazone inhibits synthesis of chlorophyll and carotenoids in plants. It is absorbed by plants through the roots from the soil and by shoots which is then translocated in the xylem and diffuses within leaves. It does not move downward in plants or from leaf to leaf. There is no foliar absorption of clomazone and is metabolized by plants [4].

Clomazone is highly water-soluble (1100 mg L^{-1}), minimally volatile ($P_v = 1.44 \times 10^{-4} \text{ mm Hg}$), resistant to hydrolysis

under a wide range of pH values and weakly sorptive to soil ($k_D = 0.47\text{-}5.30$) with sorption dependent upon organic carbon content ($k_{oc} = 300 \text{ mL g}^{-1}$). These physico-chemical characteristics indicate that it is likely to persist primarily in the water column [5]. Clomazone is highly water soluble and weakly to moderately persistent in soils with half-lives ranging from 5 to 60 days. Because of its water solubility, the potential impact on surface water, groundwater and aquatic organisms is of great concern.

Some studies on clomazone fate in soils under controlled laboratory conditions have been published in the per-reviewed literature. Clomazone degradation was affected by soil moisture, temperature, microorganisms, pH, *etc.* and sorption was influenced by organic matter, clay content and cation-exchange content [6]. Mervosh *et al.* [7] examined clomazone microbial degradation in an aerobic soil, finding slow degradation ($t_{1/2} = 49.0\text{-}58.0 \text{ d}$). Clomazone degradation was biologically dependent. Quayle *et al.* [8] conducted a field dissipation study of clomazone in water and soil in treated rice plots, finding the compound to degrade rapidly, with half-lives 7.2 d (water) and 14.2 d (soil), respectively.

However, very limited data have been reported concerning field dissipation of clomazone in soil and its terminal residues in soybean under tropical climate. With this background, the present study was conducted to find out the dissipation kinetics of clomazone applied to the soybean field under Indian tropical

condition and to assess the potential risk to the soil environment.

EXPERIMENTAL

A reference standard of clomazone (purity 95 %) and the test chemical of clomazone 50 % EC were supplied by FMC India Pvt. Ltd., Bangalore, India. All the solvents and chemicals were of analytical grade and purchased locally. For HPLC analysis, HPLC-grade methanol and 0.2 µm filtered milli-Q water were used.

Clomazone residues were determined by Agilent HPLC (1200 series) equipped with Diode Array Detector (DAD) detector, Binary pump and auto sampler with Rheodyne injection system. The separation of compounds was performed using Agilent Eclipse XDB – C 18, 5 µm, 4.6 × 150 mm column kept in thermo stated oven maintained at 35 °C. The instrument was connected to a computer which records the response in terms of peak area and height and EZchrome software was used for the acquisition of data. Methanol: water (75/25, v/v) with a flow rate of 1.0 mL min⁻¹ was used as mobile phase and the injection volume of sample was 10 µL. The detection was performed at 230 nm for all the unknown samples since the interferences were minimal at these wavelengths. The retention time of clomazone standards and samples under the above instrumental conditions was 3.10 ± 0.2 min. A calibration curve was prepared by plotting concentrations of clomazone on X-axis against the average peak area on Y-axis.

Field persistence study: A supervised field experiment was conducted at Agricultural Research Station, Tamil Nadu Agricultural University, Bhavanisagar, Tamil Nadu. The experimental farm is located in the Western Zone of Tamil Nadu at 11°29'N latitude and 77°08'E longitude with an altitude of 256 m above MSL. The experiment was laid out in randomized block design with three replications. The size of each plot was 4.5 m × 5 m. Soybean (*Glycine max* L.) variety CO (Soy) 3 was sown during 23rd November, 2011. The test chemical clomazone 50 % EC obtained from M/s FMC India Pvt. Ltd., Bangalore was applied at four different doses *viz.*, 750, 1000, 1250 and 2000 g a.i. ha⁻¹, as a pre emergence spray in soybean sown field on third day after sowing (DAS). Spray volume of 400 L ha⁻¹ was sprayed using knap-sack sprayer with flat fan nozzle. A further three replicates of plot were sprayed with water alone and maintained as control.

Collection of samples: Soil samples were collected at 0 (2 h), 1, 7, 15, 30 and 90 days after last application of clomazone and at the time of soybean harvest. About 3 kg of five-soil cores each were randomly taken from each treated and untreated plot avoiding the outer 20 cm fringes of the plots using a soil auger up to a depth of 15 cm from the surface. Pebbles and other unwanted materials were removed manually. The cores were bulked together from each plot, well mixed and stored in polythene bags at -10 °C until sample extraction. Samples from the control plots were collected before the herbicide treated plots for residue analysis. The soil was sandy clay loam in texture (clay 19.8 %, silt 9.7 % and sand 70.5 %), low in available nitrogen (234 kg ha⁻¹), medium in available phosphorus (17.7 kg ha⁻¹) and high in available potassium (265 kg ha⁻¹) with organic carbon 0.56 %, EC 0.19 dS m⁻¹ and pH 7.40.

Plant samples were collected at 0 (2 h), 1, 7, 15, 30 and 90 days after last application of clomazone. A whole plant samples and soybean grains were also collected at the time of harvest. About 500 g of representative plant samples were collected from clomazone treated and untreated plots. The plant samples were cut in small pieces and then ground on mechanical grinder and used for residue analysis. Plant samples were stored at -20 °C, until processed for residue extraction.

Weather and climate: The maximum temperature in the cropping period ranged from 29.7 to 38.9 °C with a mean of 34.4 °C. The minimum temperature ranged from 13.9 to 26.2 °C with a mean of 20.6 °C. The relative humidity ranged from 71 to 94 % with a mean of 86 % in the morning (07.22 h) and 29 to 66 % with a mean of 45 % in the evening (14.22 h). A total rainfall of 315.4 mm was received in 22 rainy days during the cropping period.

Extraction and clean-up: The clomazone residue from different matrices was extracted using methanol and water and partitioned with dichloromethane. Clean up was done using anhydrous sodium sulphate and florisil. The analyte was eluted with 2 mL petroleum ether-ethyl acetate and concentrated in a rotary evaporator [9]. The residue was re-dissolved with 2 mL methanol for HPLC analysis.

Method validation and detection limits: Validation of method was executed in terms of recovery studies before analyzing unknown samples as suggested by Janaki *et al.* [10] for oxyfluorfen in onion soil, plant and grain samples were weighed and added into extraction flasks. Required quantity of clomazone standards (0.01 to 1.0 µg mL⁻¹) was added uniformly on the surface of the matrix and mixed before adding extraction solvent. The extraction and cleanup processes were then performed as described in the methodology. Quantification of clomazone residue was accomplished by comparing the peak response for samples with peak area of the standards.

RESULTS AND DISCUSSION

Recovery of clomazone from soil and grain: Under the given conditions of HPLC, clomazone resolved at 3.10 min as a single sharp peak (Fig. 1). The limit of detection (LOD) was estimated to be 0.01 µg mL⁻¹ based on a signal to noise ratio (S/N) of 4, the limit of quantification (LOQ) of clomazone was found to be 0.05 mg/kg. The detector showed reliable sensitivity for the clomazone residues up to 0.005 µg mL⁻¹ but did not show linearity. The equations of analytical calibration graphs (Fig. 2), obtained by plotting peak areas in 'y' axis *versus* concentrations in 'x' axis within the range of 0.01 to 1.0 µg mL⁻¹ was showed good linearity and significant with the correlation coefficient of 0.997**.

The recovery experiment conducted with soybean grain, plant and soil samples showed that percentage recovery for soil varied from 89 to 103 %, however, this varied between 84 and 85 % in the case of soybean plant and 80 and 85 % in case of soybean grains at fortification level of 1 to 0.1 µg g⁻¹ of clomazone respectively (Table-1). The recoveries of clomazone from soils, soybean plant and grain at different concentration levels of 0.1 to 1 µg g⁻¹ were satisfactory being within the range of 80-103 %, confirming a reliable repeatability of the method.

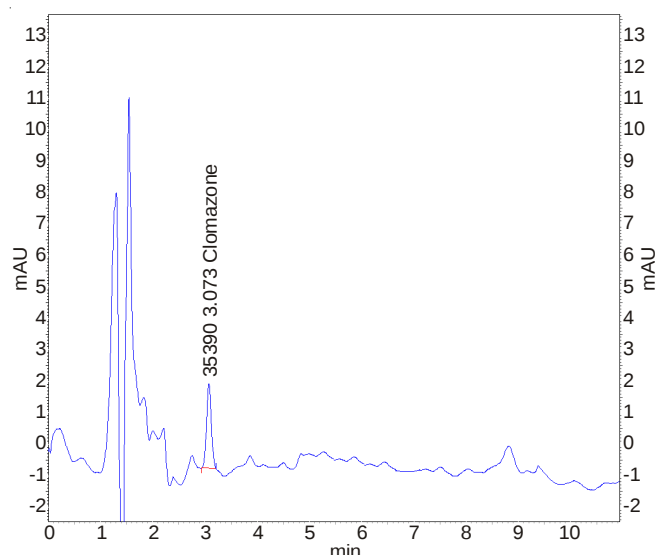


Fig. 1. Chromatogram of clomazone determined by HPLC-DAD

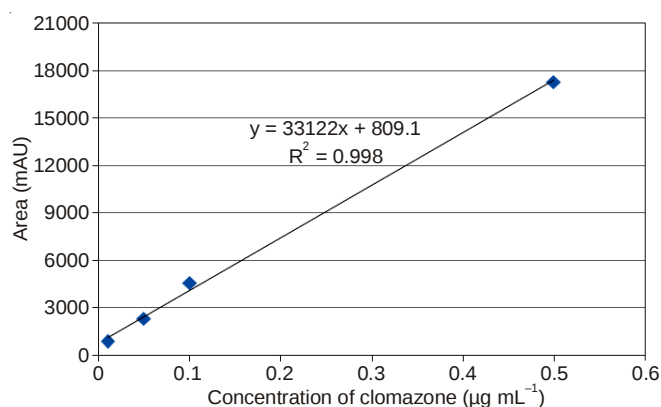


Fig. 2. Calibration curve of clomazone standards determined by HPLC-DAD

TABLE-1
RECOVERY OF THE CLOMAZONE FROM
SOIL, SOYBEAN PLANT AND GRAIN

Fortified concentration (µg g ⁻¹)	Recovery (%) * ± RSD		
	Soil	Soybean plant	Soybean grain
0.10	89.4 ± 1.82	90.1 ± 2.35	80.2 ± 3.83
0.50	96.3 ± 1.75	84.7 ± 2.38	80.5 ± 3.14
1.00	102.5 ± 0.94	85.2 ± 3.47	82.1 ± 2.89

RSD = Replication standard deviation; *Average of three replications

Persistence and dissipation in field soil: The initial concentration of clomazone in soil varied from 0.048 to 0.350 µg g⁻¹ across different doses with the highest concentration at higher dose (Table-2) of application. Clomazone dissipation in soil was faster upto 7 days, then it becomes slow and more than 80 % of the applied clomazone disappeared from the soil within 14 days irrespective of the dose of application (Fig. 3). Similar result was reported by the Hu *et al.* [9] that the clomazone dissipation rate increased to 89.6 % at the end of two weeks with the half-life of clomazone in soil 7.48 days. In the present study, the clomazone dissipation followed biphasic pattern of degradation kinetics when the log values of residue was plotted against different time intervals.

The significant correlation coefficient (Table-3) indicated statistical conformity of the dissipation data to the first order

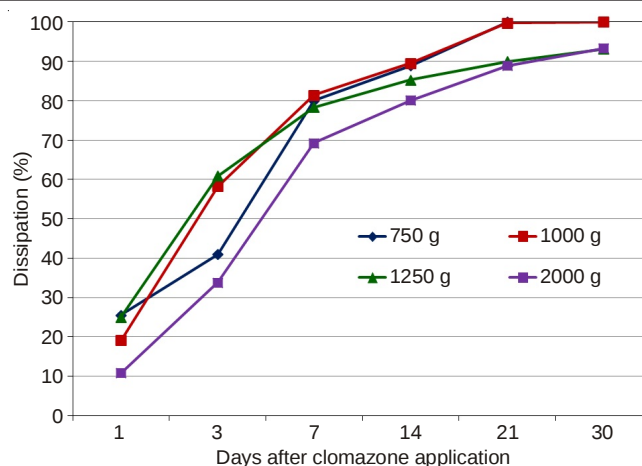


Fig. 3. Dissipation of clomazone in field soil grown with soybean

TABLE-2
PERSISTENCE OF CLOMAZONE (µg g⁻¹) IN SOIL

Days after last application	Soil (Mean* ± SD)			
	750 g ai ha ⁻¹	1000 g ai ha ⁻¹	1250 g ai ha ⁻¹	2000 g ai ha ⁻¹
0	0.0476 ± 0.0010	0.1065 ± 0.0170	0.1193 ± 0.0231	0.3503 ± 0.0380
1	0.0354 ± 0.0012	0.0861 ± 0.0035	0.0894 ± 0.0047	0.3121 ± 0.0151
3	0.0281 ± 0.0020	0.0444 ± 0.0037	0.0466 ± 0.0073	0.2318 ± 0.0107
5	0.0095 ± 0.0007	0.0198 ± 0.0019	0.0258 ± 0.0031	0.1076 ± 0.0120
7	0.0053 ± 0.0006	0.0112 ± 0.0021	0.0176 ± 0.0026	0.0696 ± 0.0078
15	BDL	BDL	0.0012 ± 0.0009	0.0389 ± 0.0057
30	BDL	BDL	0.0082 ± 0.0010	0.0234 ± 0.0034
45	BDL	BDL	BDL	BDL

SD = Standard deviation; BDL = Below detectable limit < 0.01 ppm (µg g⁻¹); *Mean of three replications.

TABLE-3
REGRESSION EQUATION, CORRELATION COEFFICIENT AND THE HALF-LIVES (t_{1/2}) OF CLOMAZONE IN FIELD SOIL

Dose	Regression equation	r ²	Half life (days)
750 g ai ha ⁻¹	y = -0.060x + 1.564	0.934	4.407
1000 g ai ha ⁻¹	y = -0.070x + 1.942	0.927	4.306
1250 g ai ha ⁻¹	y = -0.083x + 2.046	0.922	5.065
2000 g ai ha ⁻¹	y = -0.039x + 2.463	0.959	6.003

reaction kinetics. The increase in dose increased the DT₅₀ values and could be established that the fraction of the total herbicide content which was available in the soil solution influence the dissipation of it from soil [11]. Half life calculated was ranged from 4.30 to 6.00 days across different doses (Table-3). While Cumming *et al.* [12] reported the soil half-life of 6 to 59 days under a range of top soils in Australia. Such a lower half life of clomazone in the present study might be due to the leaching of it lower depths by the high rainfall received (82.5 mm) during the first week its application. Further it could be attributed to the microbial degradation and to some extent hydrolysis as reported by Mervosh *et al.* [7]. Tomco and Tjeerdema [13] also found that the soil microbial degradation to be more relevant than photolysis.

Terminal residues of clomazone: The clomazone residues in soybean grains, plant and soil collected at harvest detected by HPLC are presented in Table-4. Soil samples collected at post harvest contained 0.0014 and 0.0177 $\mu\text{g g}^{-1}$ residues where clomazone was applied at 1250 and 2000 g a.i. ha^{-1} concentrations, respectively. Clomazone residues of 0.00164 and 0.034 $\mu\text{g g}^{-1}$ were detected in soybean grains collected from 1250 and 2000 g a.i. ha^{-1} treated plots. However, 0.0037 and 0.033 $\mu\text{g g}^{-1}$ clomazone residues were found in soybean straw in 1250 and 2000 g a.i. ha^{-1} treated plots. No clomazone residues ($< 0.01 \text{ mg/kg}$) were detected in any of the matrices at the lower application rates of 750 and 1000 g a.i. ha^{-1} treatments. This could be attributed to the microbial degradation of clomazone is promoted by high soil moisture, warm temperature and optimum or neutral pH of 6.5 as reported by Vencill [4] and Mills *et al.* [14]. This would also account for the low clomazone residue concentration detected in the surface soil even at higher doses of application. Hu *et al.* [9] reported that the final residue in soybean was lower than 0.01 mg/kg at harvest time withholding a period of 3 months after herbicide application. On the basis of clomazone persistence in soil, a safe pre-harvest interval of 95 days for soybean crop after application of it is essential. The maximum residue limits (MRLs) of clomazone set by the Japan and Korea government for soybeans was 0.05 mg/kg and no maximum residue limits of the pesticide has been set by Chinese legislation or Food and Agriculture Organization/World Health Organization yet. According to maximum residue limits set by Japan and South Korea, applying clomazone in soybeans is safe [8].

TABLE-4
CLOMAZONE RESIDUES IN SOYBEAN
GRAINS, PLANT AND SOIL AT HARVEST

Dose	Clomazone residues ($\mu\text{g g}^{-1}$)		
	Soybean grain	Soybean plant	Soil
Control	BDL	BDL	BDL
750 g a.i. ha^{-1}	BDL	BDL	BDL
1000 g a.i. ha^{-1}	BDL	BDL	BDL
1250 g a.i. ha^{-1}	0.0164	0.0037	0.0014
2000 g a.i. ha^{-1}	0.0337	0.0329	0.0177

At harvest detection of low residue levels in soil may be due to adsorption of clomazone in soil due to the organic matter content and soil texture as reported by Loux *et al.* [15] that the bioavailability and degradation of clomazone in soil are functions of clomazone sorption. Loux *et al.* [16] found the

greater sorption and longer persistence of clomazone in a silty clay loam soil than in a silt loam soil.

Conclusion

On the basis of clomazone persistence in soil, a safe pre-harvest interval of 95 days for soybean after its application is suggested. Although clomazone residues were found within safe limits (0.05 mg kg^{-1}) in soil and grains at lower concentrations, residues in all the matrices were found above the safe level obtained from 1250 and 2000 g ai ha^{-1} treated plots. Thus, continuous use of clomazone in the same field needs to be avoided as this may lead to biomagnifications of residues in soil, which then bioaccumulate in crop products at higher levels of application and may enter the aquatic system through runoff, drift and leaching.

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