Bull Environ Contam Toxicol (2012) 89:312–315
DOI 10.1007/s00128-012-0697-5

Degradation of Fentrazamide Herbicide in Soil Under Aerobic Condition

Shishir Tandon · Atul Pujari · N. K. Sand

Received: 23 February 2012 / Accepted: 30 May 2012 / Published online: 12 June 2012

Abstract Dissipation of fentrazamide in soil under aerobic conditions was studied. Fentrazamide was extracted with 0.1 N HCl: acetone (1:1 v/v) followed by partition and cleanup. Separation was done on ODS-II column with mobile phase acetonitrile: water (70:30 v/v). Recovery varied from 74.51 % to 90.10 % percent in soil. Dissipation followed first order kinetics with monophasic pattern. Half life in soil was 9.02 days. Calibration curves were linear over the range of 0.05–1.00 μg mL^{-1} and RSD was 1.82 %. LOD and LOQ were 0.002 and 0.005 μg g^{-1} for soil. No residues in soil and crop were observed at harvest.

Keywords Fentrazamide · Kinetics · Dissipation · Soil · RP-HPLC

Rice (*Oryza sativa* L.) is the second largest produced cereal in the world. It is staple food for more than 60 % of the world and 70 % Indian population. Weeds, being a major threat compete with rice crop for resources and thus reducing the yield significantly. In transplanted rice, weeds causes 35 %–55 % reduction of grain yields while it is more severe in direct seeded rice where losses are above 65 %–76 % (Shekhar et al. 2004; Singh et al. 2005). With public awareness in environmental, human and animal health and resistance of certain weeds to old molecules of herbicide are causing great challenges to develop new safer herbicide which are used in low doses, highly effective and eco-friendly.

Fentrazamide [4-(2-chlorophenyl)-*N*-cyclohexyl-*N*-ethyl-4,5-oxo-1H-tetrazole-1-carboxamide] a novel new tetrazolinone/amide class herbicide developed by Bayer AG and Nihon Bayer Agro-chem with a novel chemical structure (Yanagi et al. 2001; Goto et al. 2007), used in low doses (100–300 g a.i. ha^{-1}) (Ito 2008; Chhokar et al. 2006) and have excellent efficacy as pre-emergent herbicide against grasses, annual sedges and broad leaf weeds in transplanted rice (Tomlin 2002). The selective action mechanism of fentrazamide between rice and weed species has not been clarified yet, but it acts by inhibiting seedling growth by inhibiting cell elongation and cell division (Goto et al. 2001; Böger 2003; Lim et al. 2007; Ito et al. 2008). Differential absorption, translocation, and metabolism have been recognized as important factors contributing to herbicide selectivity (Usui 2008). Studies on fentrazamide residues in soil, crop, plant, animal and multi-residue are scare (Koester 2001; Sato et al. 2001; Dubey et al. 2003; Mukherjee and Gopal 2005; Lim et al. 2008; Wang and Leung 2009; Wang et al. 2010).

No study had been reported on persistence of fentrazamide from the hot and humid sub tropical climate of Tarai region soils of Indian conditions. The registration of fentrazamide is proposed for use is under consideration by Central Insecticide Board, DPPQS, India. Thus, the present studies were undertaken to find the persistence behavior of fentrazamide in soil of Tarai region of Uttarakhand, India.

Materials and Methods

HPLC (Beckman model 322), Systronics UV–Visible Spectrophotometer Model 2101, Buchii rotavapour, SPE

S. Tandon (✉) · A. Pujari · N. K. Sand
Department of Chemistry (Division of Agricultural Chemicals), College of Basic Sciences and Humanities, G. B. Pant University of Agriculture & Technology, U. S. Nagar, Pantnagar 263 145, Uttarakhand, India
e-mail: shishir_tandon@lycos.com; shishir_tandon2000@yahoo.co.in

A. Pujari
e-mail: atulpujari@gmail.com

N. K. Sand
e-mail: nk_sand@yahoo.co.in

silica columns (6.0 × 0.75 cm id) packed with 500 mg packing material were used for estimation. Analytical grade fentrazamide (99.8 % purity) and its formulation (50 WP) were obtained from M/s Bayer AG, Germany through its Indian subsidiary Bayer (India) Ltd, Mumbai. Triple distilled water was prepared in the laboratory by double distillation of single metal distilled water in all quartz double distillation assembly.

Surface soil of 0–30 cm depth was randomly collected from five randomly selected spots (having previous history of rice growing and pesticide treatment) with the help of tube auger from the N.E. Borlaug Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar (India). Sub sample were than drawn randomly using a quartering technique, kept in air tight bags and stored in deep freeze (−20°C) until extraction. Soil was analyzed for physico-chemical properties viz., pH, CEC, Organic carbon, $CaCO_3$ percent and proportion of sand, silt and clay fraction by standard analytical procedure.

Working solutions from 0.05 to 10.0 μg mL^{-1} were prepared by serial dilution of the stock solution of 100 μg mL^{-1} with acetonitrile. Five micro liter of each concentration were injected into HPLC and peak area versus concentration was plotted. The linearity of the HPLC assay and calibration curve was evaluated in triplicate at six concentration levels ranging from 2.5 to 50 ng fentrazamide.

The operating chromatographic conditions for fentrazamide determination was Water's Spheriosorb ODS-II column, 250 mm × 4.6 mm i.d., 5 μm, the column was maintained at room temperature. Isocratic mode, mobile phase Acetonitrile: Water (70:30 v/v), the mobile phase was delivered at a flow rate 1 mL min^{-1}, column eluant was monitored in fixed wavelength UV detector at 214 nm, aufs 0.02, chart speed 1 cm min^{-1} and attenuation 10 mv. Sample injection volume was 5 μL. The mobile phase was degassed and filtered through 0.45 μm PTFE disc filter prior to use.

For recovery of fentrazamide, shade dried powdered soil 200 g of 2 mm size was taken in a glass tray and fortified with 0.05, 0.1 and 0.5 μg mL^{-1} of analytical grade fentrazamide. The soil, rice grain and straw were extracted, cleaned up and analysed by the procedure as described below.

Persistence studies was done with shade dried powdered soil of 2 mm size was placed uniformly in glass tubs (1 × 1 m) and the soil layer was wetted properly with water, allowed to equilibrate for 24 h at the ambient temperature. Pre wetted rice variety Govind were line sown at 15 cm row spacing into these tubs. Fentrazamide (50 WP) formulation was applied as pre-emergent at the rate of 120 g a.i. ha^{-1} with the help of atomizer on the soil after 3 DAS. The tubs were kept in open environment and moisture condition (≈15 %–20 %) was maintained throughout the experiment. For dissipation studies soil samples were taken by randomized sampling method at regular time interval. Paddy (grain) and straw samples were collected from all tubs at harvest time.

Fentrazamide extraction from soil (25 g) was treated with 100 mL of 0.1 N HCl: acetone (1:1 v/v) and stirred on orbital shaker for 1 h. The soil was filtered and re-extracted twice with 50 mL of 0.1 N HCl:acetone (1:1 v/v) mixture. Extracts were combined and concentrated to 5 mL under reduced pressure at 45 ± 1°C. The residue was partitioned with 50 mL dichloromethane in separatory funnel and organic layer was collected. Aqueous layer was again re-extracted twice with 25 mL dichloromethane (2 × 25 mL). The dichloromethane layer was pooled and dried over anhydrous sodium sulfate to remove traces of water. The dichloromethane fraction was filtered and evaporated to dryness under reduced pressure at 30 ± 1°C. The residue was dissolved in 1 mL dichloromethane and subjected to further clean up. The clean up was done by silica SPE. The silica SPE columns were prewashed and conditioned with 5 mL dichloromethane and the 1 mL concentrated sample was loaded on it. Column was eluted with 10 mL dichloromethane: methanol (98:2 v/v) solvent mixture. Eluent was dried under stream of nitrogen, residue obtained was dissolved in mobile phase and volume made up to 1 mL. Prior to injection of sample into HPLC it was filtered through 0.22 μm Millipore PTFE filter.

The method used for extraction and cleanup from rice straw and grain was slightly modified as given by earlier workers (Mukherjee and Gopal 2005). Rice grain (25 g) and straw (10 g) was extracted with100 mL hexane:acetone (1:1 v/v) mixture and shaked on horizontal shaker for 45 min. Same procedure was repeated twice with 50 mL solvent mixture. The solvent mixture was pooled and concentrated to 5 mL and subjected to cleanup. Cleanup was done on glass column (1.5 i.d. × 30 cm). Florisil (2 g) was sandwiched between sodium sulfate layers of 5 g each. Column was prewashed with hexane followed by dichloromethane and eluted with 100 mL dichloromethane: methanol (9:1 v/v). The eluted sample was dried under reduced pressure and residue was dissolved in HPLC grade acetonitrile.

The experiment was laid in completely randomized block design fashion and all the treatments were replicated thrice. Data were subjected to analysis to determine standard deviation among the replicates.

Results and Discussion

The physico-chemical properties revealed the texture of soil was silty loam having ratio of sand:silt:clay

Table 1 Percent recovery of fentrazamide in soil

S.No.	Amount loaded ($\mu g\ g^{-1}$)	Percent recovery		
		Soil	Rice grain	Rice straw
1	0.05	74.51 ± 0.05	78.85 ± 0.01	80.00 ± 0.03
2	0.10	80.33 ± 0.01	85.77 ± 0.05	86.33 ± 0.01
3	0.50	90.10 ± 0.02	93.33 ± 0.08	90.66 ± 0.02

Average of three replicates

(35 %:38 %:27 %), OC 0.71 %, pH 7.34, EC 0.341 and % $CaCO_3$ was 0.651. Validation of the extraction procedure of method in terms of the percent recovery after clean up from soil ranged between 74.51 % and 90.10 % at the fortification rates 0.05–0.5 $\mu g\ g^{-1}$ of soil while it was above 80 % for rice grain and straw (Table 1). The retention time was 7.9 min under operating condition. The LOD and LOQ were 0.002 and 0.005 $\mu g\ g^{-1}$ respectively. Cleanup was very efficient in removing maximum impurities extracted with the soil as there was no interfering peak at the retention time for the fentrazamide showing good specificity of the method. Detector response was linear to the concentration as the value of coefficient of determination (R^2) was 0.9881 and the percent RSD was 1.82 showing good accuracy of the method.

Amount of fentrazamide recovered from soil at different time intervals fitted in first order kinetic equation

$$C = C_0 e - \lambda^t$$

where C is amount of fentrazamide recovered from soil at time t. C_0 is amount of fentrazamide recovered at t = 0 interval, λ is degradation constant and t is time in days.

At the application rate of fentrazamide application, the natural logarithm of fentrazamide residues was plotted against time (Fig. 1). The distribution of points for soil at the levels of treatment suggested that dissipation of fentrazamide could occur through a single distinct phase conforming to first-order kinetics. The computed values of the coefficient of determination (R^2) between log residues in soil was 0.9596 (significant at $p = 0.05$), indicating that dissipation of fentrazamide could be accounted for by first-order kinetics with degradation rate constant (λ) and regression equation of 0.1029 and $Y = -0.1736x + 0.2169$, respectively. The 50 % dissipation (DT_{50}) of the herbicide was found to be 9.02 days as calculated from the slop of the regression equation. Percent persistence of fentrazamide values at different time intervals were calculated considering the amount of herbicide recovered on 0th day (1 h after application) as 100 % is shown in Table 2. The analysis of rice grain and rice straw revealed that no residues of fentrazamide were detectable at the time of harvest.

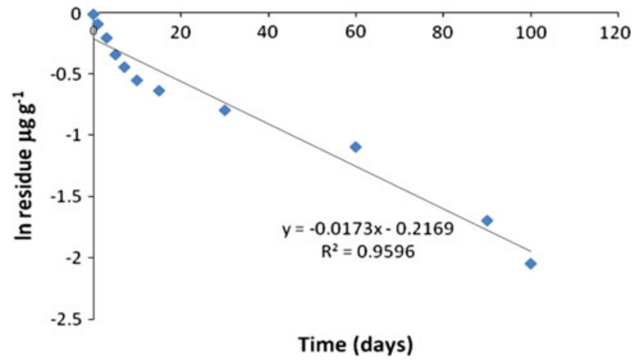

Fig. 1 Plots of natural logarithm of fentrazamide concentration in soil versus time

Table 2 Dissipation of fentrazamide in soil

Time	Amount of fentrazamide recovered ($\mu g\ g^{-1}$)	
	Amount	Dissipation
0th day (1 h)	0.96	0.00 (100)
1st day	0.80	0.16 (83.33)
3rd day	0.62	0.38 (64.58)
5th day	0.45	0.51 (46.88)
7th day	0.36	0.60 (37.50)
10th day	0.28	0.68 (29.17)
15th day	0.23	0.72 (23.95)
30th day	0.16	0.80 (16.67)
60th day	0.08	0.88 (8.34)
90th day	0.02	0.94 (2.09)
100th day	0.009	0.95 (0.94)
115th day	BDL	–
120th day (harvest)	BDL	–

BDL below detectable limits <0.005 $\mu g\ g^{-1}$

Values in parenthesis show % persistence of the herbicides

Motonga (1997) found that fentrazamide applied disappears within few days in the soil where it gets degraded and mineralized. In rice field, the residue levels in crop grain at 100 days were 0.04 mg kg^{-1} a.i. of fentrazamide thus, revealing the quick degradation of herbicide in soil and water of paddy field. Fentrazamide did not persist for long period in water and plants as it goes hydrolysis and photolysis in aqueous medium to innocuous products (Ishii 1997). Review done by workers revealed that average half of active ingredient of fentrazamide in soils disappeared with in <20 days and metabolism studies in soil and water, including aquatic systems showed the degradation pathway of a.i. to carbon dioxide (Hellpointer 2001). Major and minor metabolites of fentrazamide identified in soil was 2-chlorophenyl tetrazoline (CPT) and cyclohexyl ethyl amine (CEA) whereas, in straw and grain it was chlorophenyl tetazoline acetic acid (CPT-AA) (Morishima and Kurogochi 2001). Fentrazamide when applied at recommended (X), 2X and 4X doses were below MRL at harvest in soil, husk, rice grain and straw (Mukherjee and Gopal 2005).

Conclusion

The dissipation study of fentrazamide had revealed that herbicide undergo rapid dissipation in soil under aerobic condition. The rate of dissipation may differ in other field conditions as many other factors as temperature, organic matter, microbial flora, soil type etc. also influences the rate of degradation of the herbicide. The study also supports that herbicide fentrazamide would leave no residue in soil, rice grain and straw thereby no build up of residue in environment and safe for consumption. The methodology used for extraction, cleanup and analysis is quite rapid, simple, sensitive and specific for detection and/or quantitative analysis hence this method can reliably used for residue analysis of fentrazamide.

Acknowledgments Financial assistance and analytical grade fentrazamide and its 50 WP formulation provided by M/s Bayer AG, (Now Bayer cropsciences) Germany is duly acknowledged.

References

Böger P (2003) Mode of action for chloroacetamides and functionally related compounds. J Pestic Sci (Japan) 28(3):324–329

Chhokar RS, Sharma RK, Tripathi SC, Chauhan DS (2006) Evaluation of fentrazamide and flufenacet for weed control in transplanted rice. Ind J Weed Sci 38(3–4):230–232

Dubey PK, Mittal A, Sand NK (2003) High performance liquid chromatographic method of analysis for a herbicide YRC 2338 in soil. Pestology XXVII(7):55–57

Goto T, Ukawa S, Miyauchi H (2001) Fentrazamide—a new herbicide effective on grasses and annual sedges in rice in Japan. Pflanzenschutz Nachr Bayer. 54(1):13–34

Goto T, Yanagi A, Watanabe Y (2007) Inhibition of cell division (oxyacetamides, tetrazolinones). In: Kramer W, Schirmer U (eds) Modern crop protection compounds, vol 1. Wiley, Weinheim, pp 325–334

Hellpointer E (2001) Environmental behaviour of fentrazamide. Pflanzenschutz Nachr. Bayer 54(1):75–86

Ishii Y (1997) Metabolism of [Cyclohexyl-1 ^{14}C] YRC 2388 in rice plant. Internal report Bayer AG, Leverkusen

Ito S (2008) Study on the interaction characteristics and effects of mefenacet and fentrazamide. PhD Thesis, University of Tsukuba, Japan, p 115 (in Japanese)

Ito S, Ueno C, Goto T (2008) Effect of fentrazamide on the growth, morphology and anatomy of *Echinochloa crus-galli* and *Echinochloa oryzicola*. J Pestic Sci (Japan) 33(3):228–233

Koester J (2001) Metabolism of fentrazamide YRC 2388 in animals and plants. Pflanzenschutz Nachr Bayer 54(1):51–74

Lim SJ, Sunohara Y, Matsumoto H (2007) Action of fentrazamide on protein metabolism and cell division in plants. J Pestic Sci (Japan) 32(3):249–254

Lim SJ, Sunohara Y, Matsumoto H (2008) Absorption, translocation, and metabolism of fentrazamide in rice and early watergrass (*Echinochloa oryzicola*). Weed Biol Manag 8(3):215–218

Morishima N, Kurogochi S (2001) Residue methods and behavior of fentrazamide in plant, soil and water. Pflanzenschutz-Nachrichten Bayer (German Edition) 54(1):87–112

Motonga K (1997) Adsorption of fentrazamide (TRC2388) in soil. Internal report. Nihon Bayer Agro chem. K.K.Yuki (Japan)

Mukherjee I, Gopal M (2005) Evaluation of fentrazamide for weed control and estimation of its residues in rice. Bull Environ Cont Toxicol 74:667–672

Singh Y, Singh G, Johnson D, Mortimer M (2005) Changing from transplanted rice to direct seeding in the rice-wheat cropping system in India. In: Rice is life: scientific perspectives for the 21st century. Proceedings of the World Rice Research Conference held in Tsukuba, Japan, 4–7 November 2004, pp 198–201

Sato M, Maruyama M, Fujimura Y, Muraoka C, Yoshimoto Y, Morishima N, Kurogochi S (2001) Analytical method development for residue of fentrazamide in plant and soil. J Pestic Sci (Japan) 26:76

Shekhar J, Mankotia BS, Bindra AD (2004) Bio-efficacy of some new herbicides against weed in transplanted rice (*Oryza sativa* L). Ind J Weed Sci 36(1,2):50–53

Singh Y, Singh G, Johnson D, Mortimer M (2005) Changing from transplanted rice to direct seeding in the rice-wheat cropping system in India. In: Rice is life: scientific perspectives for the 21st century. Proceedings of the World Rice Research Conference held in Tsukuba, Japan, 4-7 November 2004. pp 198-201

Tomlin CDS (2002) The pesticide manual, 12th edn. British Crop Protection Council BCPC publication, UK, pp 406–407

Usui K (2008) Metabolism and selectivity of rice herbicides in plants. Weed Biol Manag 1(3):137–146

Wang J, Leung D (2009) Determination of 142 pesticides in fruit and vegetable-based infant foods by liquid chromatography/electrospray ionization-tandem mass spectrometry and estimation of measurement uncertainty. J AOAC Int 92(1):279–301

Wang J, Leung D, Chow W (2010) Applications of LC/ESI-MS/MS and UHPLC QqTOF MS for the determination of 148 pesticides in berries. J Agric Food Chem 58(10):5904–5925

Yanagi A, Watanabe Y, Narabu S, Ito S, Goto T (2001) Synthesis and herbicidal activity of 1-(sub.) phenyl-5-carbamoyltetrazolinone derivatives: synthesis of fentrazamide. J Pestic Sci (Japan) 26:62