

Full Length Research Paper

Influence of pH on persistence and dissipation of clofentezine, a tetrazine acaricide in water

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Clofentezine is an environment friendly and integrated pest management (IPM) compatible pesticide. It is an acaricide that is used in plant protection products for the control of spider mites on a wide range of crops. It acts primarily as an ovicide, but has some activity against early motile stages of mites. In the present research, dissipation behavior of Clofentezine in acidic, neutral and alkaline water was studied. Double distilled buffered water of pH 4.0, 7.0 and 9.2 (200 ml) was spiked at two treatment doses at 1.0 and 2.00 µg/ml. Samples were withdrawn at different time intervals and extracted with a mixture of hexane/dichloromethane and analyzed by high pressure liquid chromatography (HPLC). The results of the study indicated that degradation of Clofentezine in water is highly pH dependent. Clofentezine undergoes a base catalyzed hydrolysis resulting in lowest persistence in alkaline water and highest in acidic water. The half-life values were in the range of 0.53 to 60 days.

Key words: Clofentezine, water, pH, persistence, half-life.

INTRODUCTION

Investigating pesticide degradation occurring in the environment is of high interest because as the parent compound, the decomposition products can also be toxic and hazardous. Many researchers have studied acaricides degradation (Barba et al., 1991; Wang et al., 1994; Hatzilazarou et al., 2004; Sharma et al., 2008; Juraske et al., 2008; Kumar et al., 2005).

Clofentezine [3,6-bis-(2-chlorophenyl)-1,2,4,5-tetrazine] is an acaricide of novel structure belonging to tetrazine group. The compound is registered in several countries for use in agriculture for the protection of food and non food crops, ornamentals and in orchards. It interferes with cell growth and differentiation during the final stages of embryonic and early larval development [Food and Agriculture Organization (FAO, 2000) Specification]. The

compound has been shown to be active against the eggs and immature stages but does not kill the adults (Aveyard et al., 1986). Efficacy of the compound in controlling *Panonychus* spp., *Tetranychus* spp., *Rhizoglyphus* spp. has been reported (Welty et al., 1989; Helm, 1989; Wu and Lo, 1990; Zhao et al., 1996; Li et al., 2006; Hardman et al., 2007). Clofentezine is an environment friendly and integrated pest management (IPM) compatible pesticide. Fast dissipation of the compound after the field application has been reported by Gonzalez and Aravena (1990), Akashe et al. (2002), and Urbaneja et al. (2008). Residues of Clofentezine can be determined by high pressure liquid chromatography (HPLC) with ultraviolet detection (Barnes et al., 1995; Navickiene and Ribeiro, 2004; Tuzimski, 2008), thin layer chromatography (TLC)

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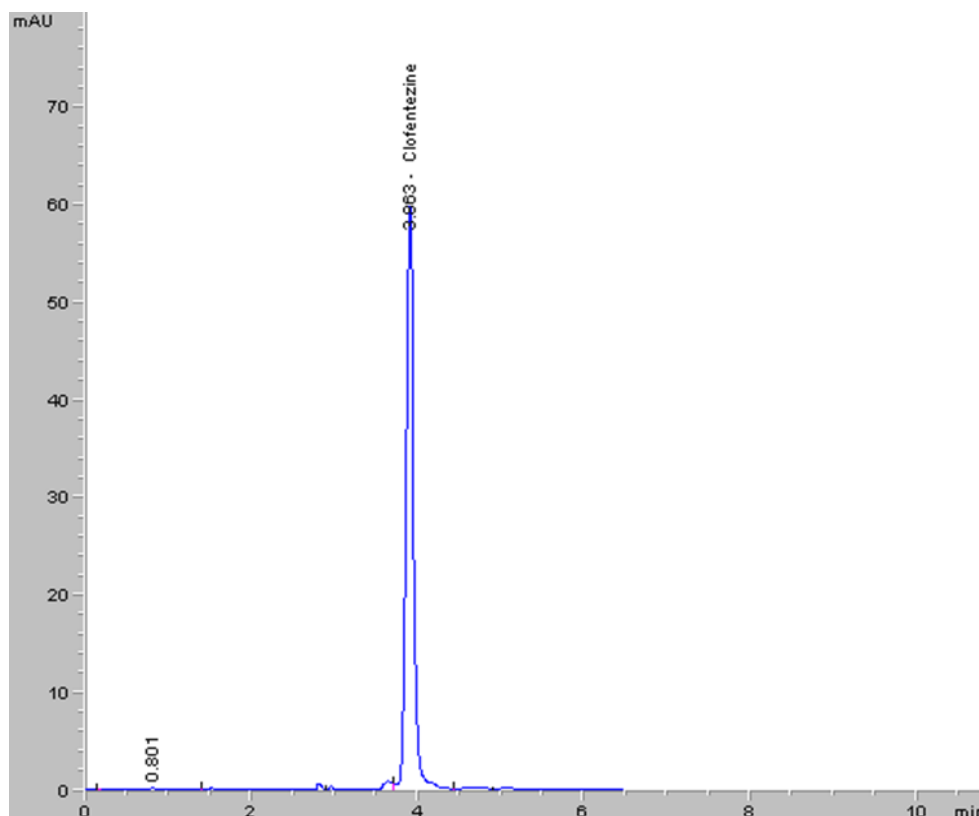


Figure 1. HPLC chromatogram of Clofentezine (5 µg/ml).

(Balinov and Balinova, 1999) and gas chromatography-mass spectrometry detector (GC-MSD) (Snowdon et al., 1991; Menezes et al., 2010). In the present study, persistence behavior of Clofentezine in water had been studied. The effect of pH on the dissipation behavior was also evaluated.

MATERIALS AND METHODS

Three Erlenmeyer flasks (250 ml) were filled with 200 ml of double distilled water. Appropriate buffer capsules (Merck) were added (one capsule/100 ml) to bring the pH of the water at 4.0, 7.0 and 9.2.

Clofentezine analytical standard solution (100 µg/ml) was used to spike the water at two treatment doses at 1.0 µg/ml (T_1) and 2.00 µg/ml (T_2). After application the solutions were stirred for 2 min and covered with perforated aluminum foil. The Erlenmeyer flasks were incubated in the dark at 25°C. Samples were withdrawn at 0, 1, 3, 7, 15, 30, 45, 60, 90 and 120 days after application for pH 4.0. For pH 7.0, samples were withdrawn at 0, 1, 3, 7, 15, 30, 45 and 60 days after application. For pH 9.2 sampling intervals were 0, 12, 24, 36, 48, 72, 120, 240, 360 and 720 h after application.

Water samples were partitioned with hexane: dichloromethane: 1:1 (v/v) mixture (100, 50, and 50 ml). The organic layer was collected by passing through anhydrous Na_2SO_4 (E Merck, India) and dried in the rotary vacuum evaporator. The residue was then transferred to measuring tubes with HPLC grade acetonitrile for HPLC analysis. The final volume was made up to 200 ml for T_1 and 400 ml for T_2 .

The residues of Clofentezine were estimated by HPLC using Agilent 1200 series HPLC equipped with ultraviolet-visible (UV-VIS) detector and ODS Hypersil column (250 × 4.6 mm). The detector was set at 220 nm wavelength. The solvent system used was Acetonitrile: Water (90:10) at 1 ml/min flow rate. The retention time was 3.863 min (Figure 1).

The method was validated by standard calibration curve and accuracy (recovery) experiment. The calibration curve was obtained by plotting the peak areas against the concentration of the standard solution. The limit of detection (LOD) of Clofentezine was determined by considering a signal-to-noise ratio (S/N) of 3 with reference to the background noise obtained for the blank sample, whereas the limits of quantification (LOQ) were determined by considering an S/N of 10, according to the peak-to-peak noise method as proposed by US Environmental Protection Agency (EPA) (Corley, 2003).

Recovery study was carried out in order to establish the authenticity of the analytical method employed. Water samples (200 ml) were fortified at the level of LOQ and 10 times of LOQ with the analytical standard solutions of Clofentezine and were analyzed following the above procedure. Five samples were taken at each fortification level along with two control samples.

In order to explain the nonlinear dissipation pattern of Clofentezine in water a first order model was adopted. The residue data of Clofentezine in water was analyzed using the following mathematical expression:

$$[A]_t = [A]_0 \exp^{-kt}$$

Where $[A]_t$ is the concentration (mg/L) of A at time t (days) and $[A]_0$ is initial concentration of A at time 0 (days) and k is the degradation

Table 1. Recovery study of Water samples spiked with analytical standard of Clofentezine.

pH level	Amount fortified ($\mu\text{g/ml}$)	Amount recovered ($\mu\text{g/ml}$)*	% recovery	RSD
4.0	0.01	0.0905	90.5	12.6
	0.10	0.9260	92.6	10.4
7.0	0.01	0.0915	91.5	11.4
	0.10	0.9240	92.4	9.7
9.2	0.01	0.0908	90.8	12.5
	0.10	0.9420	94.2	8.7

*Average of five replicates; RSD = Relative standard deviation.

rate constant. However, a more simplified differential form of the above equation was employed to obtain dissipation parameters. This is actually a linear plot of logarithmic transformation of the residues versus time; each residue value being multiplied by 1000 and log value calculated. The half-life values (DT_{50}) in water were then evaluated applying the following relation:

$$DT_{50} = \ln(2) \times k^{-1}$$

RESULTS AND DISCUSSION

The linearity of the calibration curve was established in the range 0.1 to 10 $\mu\text{g/ml}$ with a correlation coefficient $R^2 > 0.99$. The LOD and LOQ were 0.004 and 0.01 $\mu\text{g/ml}$, respectively. Average recovery percentages from the fortified samples of water at different pH ranged between 90 and 94% with relative standard deviations (RSD) from 8.7 to 12.6 (Table 1). Control samples were free of Clofentezine residues. Thus, there was compliance with the EU DG SANCO criterion in this regard, which requires mean recoveries within the range of 70 to 110% (SANCO, 2004).

Clofentezine is varyingly persistent in water of different pH. Its persistence is highly dependent upon pH of the water. At pH 9.2, the initial concentrations of Clofentezine in water were 0.96 and 1.92 $\mu\text{g/ml}$ for T_1 and T_2 (Table 2). Thereafter, the residue was found to decrease gradually with time recording >90% dissipation by 2 and 10 days in T_1 and T_2 doses, respectively and reached below quantification level (BQL) after 5 and 30 days after application. The residues degraded very fast following a first order kinetics (Figure 2) with a half-life of 0.5 to 2.7 days (Table 5).

At pH 7.0, the initial concentrations of Clofentezine in water were 0.96 and 1.97 $\mu\text{g/ml}$ for T_1 and T_2 , respectively (Table 3). The residues decreased by more than 50% after 15 days, more than 80% after 30 days, more than 90% after 45 days and reached BQL after 60 to 90 days after application. Degradation kinetics is shown graphically (Figure 3). The half-life values for T_1 and T_2 were found to be 9.9 and 12.3 days, respectively (Table 5).

A relatively higher persistence of Clofentezine was obtained in water at pH 4.0, in comparison to pH 7.0 and pH 9.2. For T_1 and T_2 , the corresponding initial concentration levels were 0.99 and 1.98 $\mu\text{g/ml}$ (Table 4). The initial concentration decreased by about 50% after 45 and 60 days in T_1 and T_2 doses, respectively which further increased by more than 75% after 120 days of application. The dissipation followed first order kinetics and the linear plot is presented graphically (Figure 4). Regression equations for the first order kinetics along with half-life values (49.3 to 60.2 days) are given in Table 5.

From the results of persistence study of Clofentezine in water at acidic, neutral and alkaline condition, it appeared that degradation of the compound in water is highly pH dependent. Clofentezine undergoes a base catalyzed hydrolysis resulting in lowest persistence in alkaline water and highest in acidic water. The half-life values were in the range of 0.53 to 60 days. The dissipation behavior reflects wide variations in persistence of the compound in water at different pH levels.

From the findings of the study, it may be concluded that Clofentezine, the tetrazine acaricide is prone to alkaline hydrolysis. Its persistence in aquatic condition is related with the pH of water. Any unwanted contamination of water with Clofentezine through accidental spillage, runoff and indiscriminate use or by any other means can be easily addressed by simply altering the pH of the water. Similar types of findings were also reported by Canada Pesticide Directorate (1989) in the Decision document on Clofentezine. It was reported that the compound undergoes base-catalyzed hydrolysis. The half-lives for hydrolysis were reported to be approximately 4 h at pH 9, 34 h at pH 7 and 249 h (10 days) at pH 5.

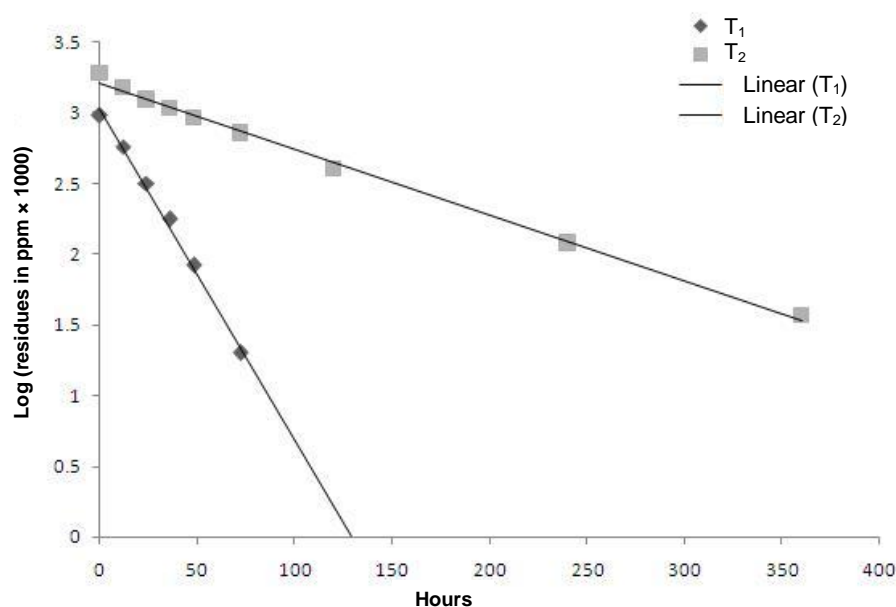
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Table 2. Residues of Clofentezine in alkaline water.

Hour	Residues in $\mu\text{g/ml}$ (Mean \pm SD) (Dissipation %)	
	T ₁	T ₂
0	0.96 \pm 0.010	1.92 \pm 0.020
12	0.58 \pm 0.035 (39.33)	1.51 \pm 0.031 (21.53)
24	0.32 \pm 0.015 (67.01)	1.24 \pm 0.040 (35.24)
36	0.18 \pm 0.025 (81.60)	1.08 \pm 0.040 (43.75)
48	0.08 \pm 0.015 (91.32)	0.92 \pm 0.021 (52.26)
72	0.02 \pm 0.010 (97.92)	0.72 \pm 0.036 (62.50)
120	BQL	0.40 \pm 0.021 (78.99)
240		0.12 \pm 0.030 (93.75)
360		0.037 \pm 0.006 (98.09)
720		BQL

BQL, Below quantification limit.

**Figure 2.** Linear plot for first order kinetics of clofentezine in alkaline water.**Table 3.** Residues of Clofentezine in neutral water.

Day	Residues in $\mu\text{g/ml}$ (Mean \pm SD) (Dissipation %)	
	T ₁	T ₂
0	0.96 \pm 0.032	1.97 \pm 0.031
1	0.85 \pm 0.015 (11.42)	1.84 \pm 0.040 (6.27)
3	0.76 \pm 0.015 (20.76)	1.68 \pm 0.040 (14.75)
7	0.59 \pm 0.021 (39.10)	1.29 \pm 0.026 (34.41)
15	0.38 \pm 0.031 (60.90)	0.98 \pm 0.040 (50.34)
30	0.12 \pm 0.020 (87.54)	0.35 \pm 0.031 (82.37)
45	0.04 \pm 0.010 (95.85)	0.15 \pm 0.010 (92.37)
60	BQL	0.07 \pm 0.020 (96.44)
90		BQL

BQL, Below quantification limit.

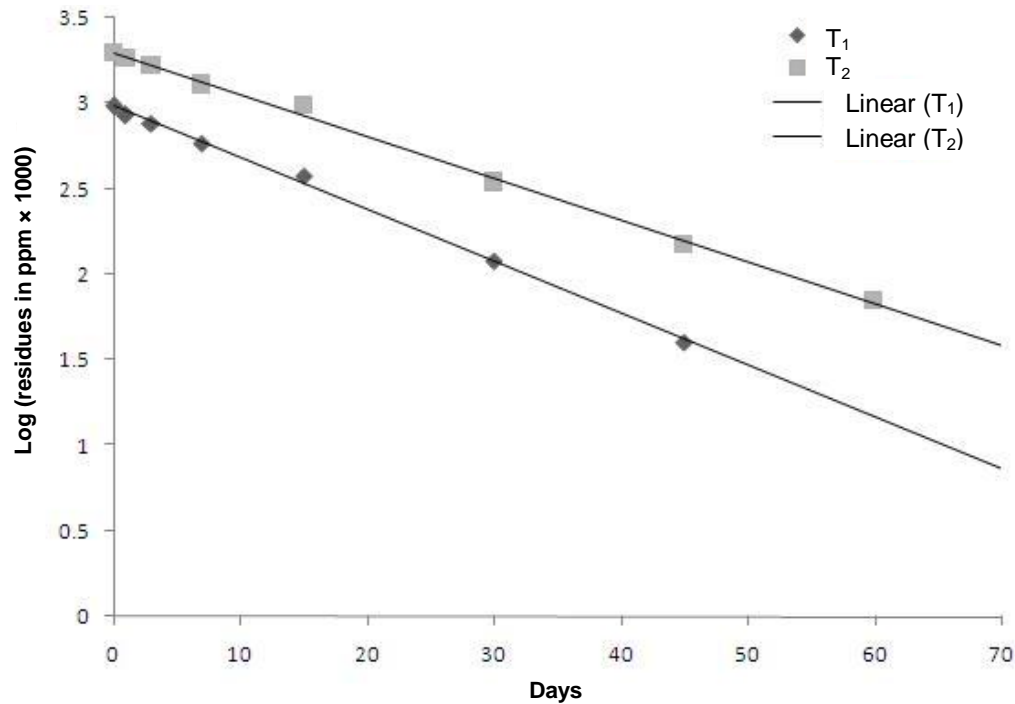


Figure 3. Linear plot for first order kinetics of clofentezine in neutral water.

Table 4. Residues of Clofentezine in acidic water.

Day	Residues in $\mu\text{g/ml}$ (Mean \pm SD) (Dissipation %)	
	T ₁	T ₂
0	0.99 \pm 0.017	1.98 \pm 0.025
1	0.92 \pm 0.015 (7.41)	1.88 \pm 0.025 (5.06)
3	0.85 \pm 0.020 (14.14)	1.77 \pm 0.021 (10.29)
7	0.82 \pm 0.025 (16.84)	1.65 \pm 0.025 (16.69)
15	0.76 \pm 0.021 (23.57)	1.59 \pm 0.021 (19.73)
30	0.62 \pm 0.025 (37.04)	1.37 \pm 0.021 (30.86)
45	0.49 \pm 0.020 (50.51)	1.17 \pm 0.021 (40.64)
60	0.41 \pm 0.021 (58.25)	0.99 \pm 0.038 (49.58)
90	0.28 \pm 0.026 (71.72)	0.70 \pm 0.025 (64.76)
120	0.16 \pm 0.015 (83.50)	0.46 \pm 0.015 (76.90)

Table 5. Regression equation and half-life of Clofentezine in water maintained at different pH.

Water pH	Treatment	Regression equation	Half-life, DT ₅₀
pH 9.2	T ₁	$y = 3.0368 - 0.0235x$	0.53 days (12.81 h)
	T ₂	$y = 3.2131 - 0.0047x$	2.67 days (64.04 h)
pH 7.0	T ₁	$y = 2.9835 - 0.0303x$	9.93 days
	T ₂	$y = 3.2981 - 0.0245x$	12.29 days
pH 4.0	T ₁	$y = 2.9713 - 0.0061x$	49.34 days
	T ₂	$y = 3.2785 - 0.0050x$	60.2 days

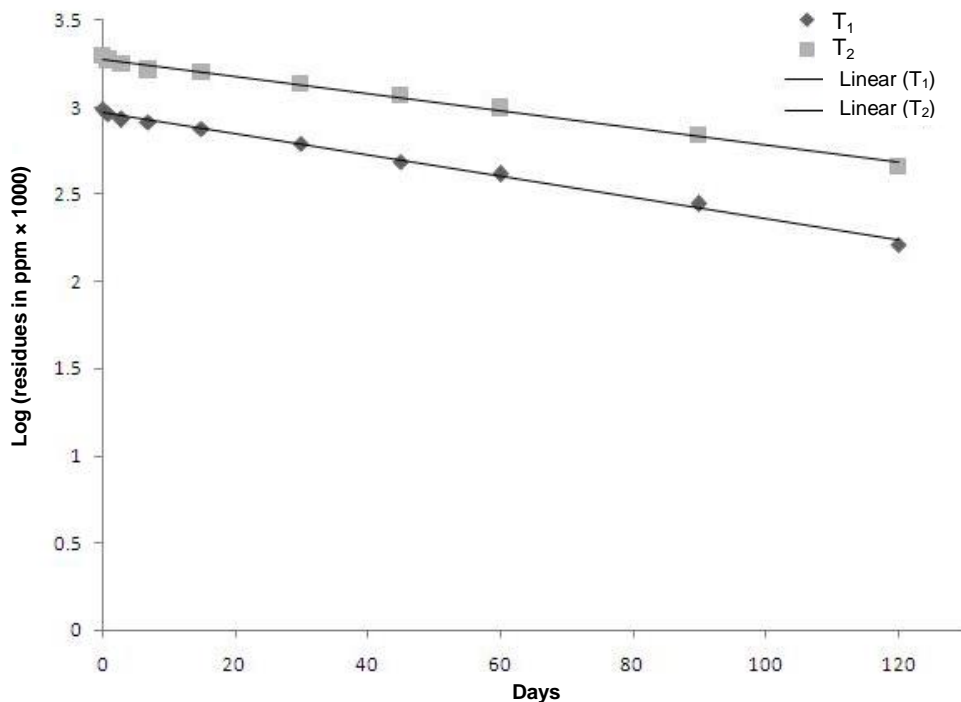


Figure 4. Linear plot for first order kinetics of clofentezine in acidic water.

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