CHAPTER-5

Investigation of Effect of pH on Dissipation of Tebuconazole in aqueous medium

5.1 Introduction

Pesticide residue from soil can reach water bodies by leaching and runoff (Frank, R. *et al*., 1987). The present study has been designed to investigate the dissipation/degradation behavior of tebuconazole in the sterile water at pH 4, 7 and 9. The rate of hydrolysis and half-life of tebuconazole in water at pH 4.0, 7.0 and 9 was determined at temperature 30 ± 2 ºC. The dissipation study in water with wide range of pH would also enable us to understand tebuconazole interaction with ions and inorganic micelles (clays) of soils. The test was performed using the, European commission guideline EC, C.7 (EC, 1997).

5.2 Materials and Methods

5.2.1 Chemicals

- Ethyl acetate and anhydrous sodium sulphate (AR grade) was purchased from Merck, India.
- The tebuconazole standard (purity 99.24%) was supplied by United Phosphorus Ltd (UPL) Mumbai, India.
- Sodium hydroxide, monopotassium citrate, monopotassium phosphate, boric acid and potassium chloride were purchase from Ranchem and s.d Fine chemicals, India

5.2.2 GC and Operating Conditions

Capillary gas chromatograph (GC) equipped with nitrogen-phosphorous selective detector (NPD) and split mode injector (Perkin Elmer, USA, and Model, Clarus 500) was used for analysis of tebuconazole residue in water. The operation conditions are given below,

Column	:	Dura Bond mid-polar fused-silica capillary with low bleed (DB-35 column: packing with 35% Phenyl-methyl polysiloxane,
Column dimension	:	30 m × 0.25 mm I.D., 0.25 micron film thickness)
Instrument software	:	Total Chrom

Instrument operating temperature parameters;

Column Oven	:	200 to 275 °C @ 25 °C/min (hold for 8.0 min)
Injector port	:	260 °C
NPD Detector	:	290 °C
Carrier gas and its flow rate	:	Helium and 1.0 ml/min
Hydrogen gas flow rate	:	1.5 ml/min
Air flow rate	:	95 ml/min
Injected Volume	:	2µl
Split ratio	:	vent: column, 2:1

- Ultrasonic bath (Fixed Frequency 35 kHz, Spectra lab, India) and Centrifuge (Rota 4RV/FM, Plasto Crafts, India) was used for sonication and centrifugation of samples. The Autoclave was used for sterilization and biological oxygen demand (BOD) (Somax, India) used for incubation of water samples.

5.2.3 Preparation of Solutions

Buffer (pH 4) The buffer solution (pH 4) was prepared by mixing 90.0 ml 0.1 N sodium hydroxide solution with 500 ml 0.1M monopotassium citrate solution in a 1000 ml volumetric flask and the volume was made up to 1000 ml using sterile distilled water.

Buffer (pH 7) The buffer solution (pH 7) was prepared by mixing 296.3 ml 0.1 N sodium hydroxide solution with 500 ml 0.1 M monopotassium phosphate solution in a 1000 ml volumetric flask and the volume was made up to 1000 ml using sterile distilled water.

Buffer (pH 9) The buffer solution (pH 9) was prepared by mixing 213.0 ml 0.1 N sodium hydroxide solution with 500 ml 0.1 M boric acid in 0.1 M potassium chloride solution in a 1000 ml volumetric flask and the volume was made up to 1000 ml using sterile distilled water.

The buffer solutions were sterilized by autoclaving at 121 ºC for 20 min and passing through 0.2 µm membrane filter, using sterilized glass wares under aseptic condition in a laminar flow chamber. The pH of buffer solution was checked using pH meter.

Tebuconazole Sample Solution

A quantity of 250 mg tebuconazole (99.24% purity) was weighed and transferred into a 500 ml capacity of volumetric flask (sterilized) containing 5 ml ethyl acetate, the contents dissolved and volume was made up to the mark with sterile distilled water. The concentration of standard stock solution was 500 µg/ml.

5.2.4 Recovery studies

Recovery studies were carried out by spiking of water sample of pH 4.0, 7.0 and 9.0 with sample solution of concentration levels 4 µg/ml and 8µg/ml separately. The fortified water samples of pH 4, 7 and 9 were extracted and recovery was determined by capillary GC-NPD technique.

The flasks containing water sample were placed in ultrasonic bath (Frequency of 35 kHz) at temperature 30 °C ± 2 for 15 min sonication then the contents was transferred into centrifuge tubes and centrifuged for 10 min at 4000 rpm. The organic

layer was separated and the moisture from the extracted organic layer was removed by passing through sufficient amount of sodium sulphate and the extracts were clean by filtered through 0.2 µm PTFE syringe filter. The filtrates were dried under gentle stream of nitrogen gas. The residues were re-constituted with 1 ml ethyl acetate, from that 2 µl of aliquot was injected into capillary GC-NPD for analysis.

The mean recoveries in water at pH 4.0,7.0 and 9.0 for spiked concentration levels 4 µg/ml and 8 µg/ml were found in the range of 98.2-98.4%, 99.08-99.45% and 99.08 -99.45% respectively.

5.2.5 Spiking and Incubation of water Sample at pH 4, 7 and 9

The 50 ml of sterile water at pH 4, 7 and 9 were spiked in triplicate at treatment level 4 µg/ml (Treatment, T1) and 8 µg/g (T2) separately. Experiments were performed in separate flasks for each water type for time periods 0 (2 hours after spiking), 3, 7, 15, 30, 45, 60, 75, 90 and 120 days separately. One flask for each water pH as per each sampling interval was kept as control.

No. of aqueous medium = 3 (pH 4, pH7 and pH 9)

Application rates = 2 (T1- 4 µg/ml and T2-8 µg/g)

3 replicate experiments for each water pH at each application rate

5.2.6 Extraction and Cleaning of Samples

After the desired time interval, the water sample containing flask was placed in ultrasonic bath (Frequency of 35 kHz) at 30 °C temperature for 15 min. The contents was transferred into centrifuge tubes and centrifuged for 10 min at 4000 rpm. The organic layer was separated; moisture from the extracts were removed by passing through sufficient amount of sodium sulphate and the extracts were clean by filtered through 0.2 µm PTFE syringe filter, the filtrates were dried under gentle stream of

nitrogen gas. The residues were re-constituted with 1 ml ethyl acetate; from which 2 µl of aliquot was injected into capillary GC-NPD for analysis.

The computation of residue data for determination of dissipation and half-lives of tebuconazole in water at different pH was done as described in chapter 4 for soil.

5.2.7 Monitoring of Sterility

Spread plate counting method was followed for monitoring the sterility of the samples on every sampling day. Readymade nutrient agar media [composition : peptic digest of animal tissue (5.00 g/L), sodium chloride (5.00 g/L), beef extract (1.50 g/L), yeast extract (1.50 g/L) and agar (15.00 g/L)] was used. The media was prepared by 4.2 g nutrient agar in 150 ml distilled water. The Petridis, nutrient agar media, and glassware were sterilized by autoclaving at 121 ºC for 15 minutes. The sterilized nutrient agar media was poured into the Petri dish and allowed to solidify. Then three drops of test solution taken from the respective flask using sterilized pipette was added to the surface of the solidified media and spread with sterilized glass spreader. The plates were incubated at 37 ± 2 ºC in BOD incubator. The observations were recorded at 24-hours interval up to 48 hours.

5.3 Results and Discussion

The percent dissipation and half-life values of tebuconazole in water at different pH are presented in Table 5.1 to 5.3. At spiked concentration levels 4 µg/ml and 8 µg/ml, the initial concentration of tebuconazole ranged between 3.928 – 7.872 µg/ml, 3.963 – 9.956 µg/ml and 3.982 – 7.926 µg/ml for pH 4.0, 7.0 and 9.0 respectively. After 120 days, the residue remaining in water of pH 4.0, 7.0 and 9.0 ranged between 3.128 – 6.268 µg/ml, 3.198 – 6.388 µg/ml and 3.203 – 6.335 µg/ml respectively.

Tebuconazole is more stable under neutral and basic conditions. At pH 7.0 only 19.3 – 19.71 % got dissipated even after 120 days while at pH 9.0, the percentage dissipation varied from 19.56 – 20.07%. Only under acidic condition (at pH 4.0) the compound showed significant dissipation of 20.38 % after 120 days (Table 5-1- 5.3).

Table 5.1: Dissipation of tebuconazole in water at pH 4.0 under laboratory condition

Treatment	Time Period (Day)	Residue (µg/ml)			Mean ± SD	Percentage Dissipation
Tebuconazole (4µg/ml)	0	3.923	3.921	3.939	3.928 ± 0.008	–
	3	3.886	3.869	3.879	3.878 ± 0.007	1.27
	7	3.809	3.805	3.825	3.813 ± 0.009	2.93
	15	3.738	3.735	3.749	3.741 ± 0.006	4.76
	30	3.652	3.647	3.665	3.655 ± 0.008	6.95
	45	3.565	3.556	3.568	3.563 ± 0.005	9.29
	60	3.477	3.455	3.462	3.465 ± 0.009	11.79
	75	3.369	3.349	3.358	3.359 ± 0.008	14.49
	90	3.245	3.245	3.256	3.249 ± 0.005	17.29
	120	3.144	3.119	3.121	3.128 ± 0.011	20.37
	Regression equation: $y = 3.58889-0.00082x$; $r^2 = 0.992$; Half-life ($t_{1/2}$) = 367.25 days					
Tebuconazole (8µg/ml)	0	7.878	7.87	7.868	7.872 ± 0.004	–
	3	7.732	7.726	7.723	7.727 ± 0.004	1.84
	7	7.586	7.576	7.569	7.577 ± 0.007	3.75
	15	7.421	7.407	7.405	7.411 ± 0.007	5.86
	30	7.235	7.229	7.235	7.233 ± 0.003	8.12
	45	7.054	7.043	7.046	7.048 ± 0.005	10.47
	60	6.862	6.854	6.851	6.856 ± 0.005	12.91
	75	6.664	6.653	6.651	6.656 ± 0.006	15.45
	90	6.457	6.453	6.452	6.454 ± 0.002	18.01
	120	6.277	6.263	6.265	6.268 ± 0.006	20.38
	Regression equation: $y = 3.88676-0.00081x$; $r^2 = 0.978$; Half-life ($t_{1/2}$) = 371.6 days					

Table 5.2: Dissipation of tebuconazole in water at pH 7.0 under laboratory condition

Treatment	Time Period (Day)	Residue (µg/ml)			Mean ± SD	Percentage Dissipation
Tebuconazole (4µg/ml)	0	3.974	3.959	3.955	3.963 ±0.008	–
	3	3.908	3.915	3.9	3.908 ±0.006	1.39
	7	3.854	3.845	3.842	3.847 ±0.005	2.93
	15	3.779	3.768	3.771	3.773 ±0.005	4.79
	30	3.699	3.688	3.685	3.691 ±0.006	6.86
	45	3.606	3.593	3.595	3.598 ±0.006	9.21
	60	3.508	3.498	3.497	3.501 ±0.005	11.66
	75	3.404	3.395	3.394	3.398 ±0.004	14.26
	90	3.302	3.285	3.287	3.291 ±0.008	16.96
	120	3.208	3.192	3.194	3.198 ±0.007	19.3
	Regression equation: $y = 3.5919-0.00078x$; $r^2 = 0.986$; Half-life ($t_{1/2}$) = 385.9 days					
Tebuconazole (8µg/ml)	0	7.956	7.951	7.962	7.956 ±0.005	–
	3	7.864	7.865	7.872	7.867 ±0.004	1.12
	7	7.764	7.762	7.775	7.767 ±0.006	2.38
	15	7.641	7.638	7.645	7.641 ±0.003	3.96
	30	7.508	7.505	7.512	7.508 ±0.003	5.63
	45	7.359	7.348	7.345	7.351 ±0.006	7.6
	60	7.157	7.155	7.165	7.159 ±0.004	10.02
	75	6.949	6.941	6.949	6.946 ±0.004	12.69
	90	6.703	6.691	6.689	6.694 ±0.006	15.86
	120	6.396	6.386	6.383	6.388 ±0.006	19.71
	Regression equation: $y = 3.89834-0.00077x$; $r^2 = 0.994$; Half-life ($t_{1/2}$) = 390.91 days					

Table 5.3: Dissipation of tebuconazole in water at pH 9.0 under laboratory condition

Treatment	Time Period (Day)	Residue (µg/ml)			Mean ± SD	Percentage Dissipation
Tebuconazole (4µg/ml)	0	3.989	3.979	3.978	3.982 ±0.005	–
	3	3.929	3.926	3.925	3.927 ±0.002	1.38
	7	3.873	3.859	3.857	3.863 ±0.007	2.99
	15	3.781	3.778	3.785	3.781 ±0.003	5.05
	30	3.702	3.69	3.687	3.693 ±0.006	7.26
	45	3.601	3.594	3.593	3.596 ±0.004	9.69
	60	3.497	3.493	3.495	3.495 ±0.002	12.23
	75	3.402	3.386	3.382	3.39 ±0.009	14.87
	90	3.288	3.279	3.276	3.281 ±0.005	17.6
	120	3.212	3.198	3.198	3.203 ±0.007	19.56
	Regression equation: y=3.59327-0.0008x; r^2 = 0.981; Half-life ($t_{1/2}$) = 376.25 days					
Tebuconazole (8µg/ml)	0	7.934	7.923	7.922	7.926 ±0.005	–
	3	7.841	7.828	7.83	7.833 ±0.006	1.17
	7	7.722	7.708	7.703	7.711 ±0.008	2.71
	15	7.582	7.573	7.575	7.577 ±0.004	4.4
	30	7.439	7.435	7.437	7.437 ±0.002	6.17
	45	7.284	7.268	7.263	7.272 ±0.009	8.25
	60	7.096	7.084	7.082	7.087 ±0.006	10.59
	75	6.89	6.875	6.872	6.879 ±0.008	13.21
	90	6.637	6.627	6.63	6.631 ±0.004	16.34
	120	6.345	6.33	6.329	6.335 ±0.007	20.07
	Regression equation: y=3.89514-0.00078x; r^2 = 0.993; Half-life ($t_{1/2}$) = 385.9 days					

The half-life values were calculated from the best-fit lines (Figures 5.1 – 5.3), suggested first order dissipation kinetics ($r^2 > 0.97$) for tebuconazole dissipation in water at different pH. The half-life values were 367.25 – 371.6 days for pH 4.0, 385.9 – 390.91 days for pH 7.0 and 376.25 – 385.9 days for pH9.0. The rate of tebuconazole dissipation was found slightly more in pH 4.0 followed by pH 9.0 and pH 7.0, thereby, indicating the stability of the molecule in neutral and basic pH.

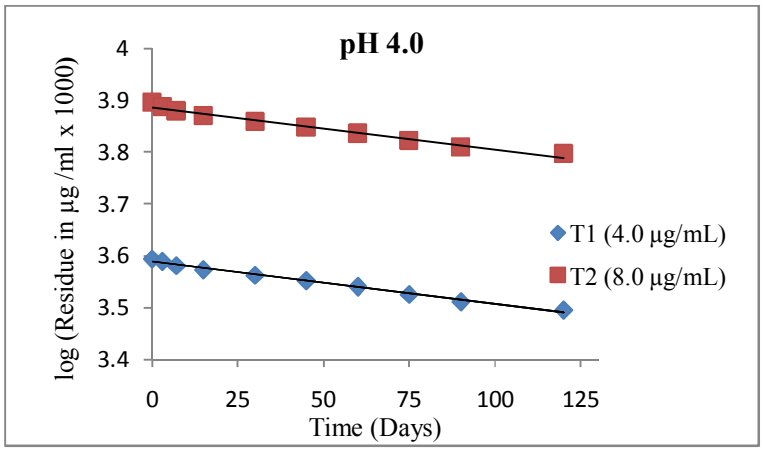

Figure 5.1: Dissipation of tebuconazole in water at pH 4.0

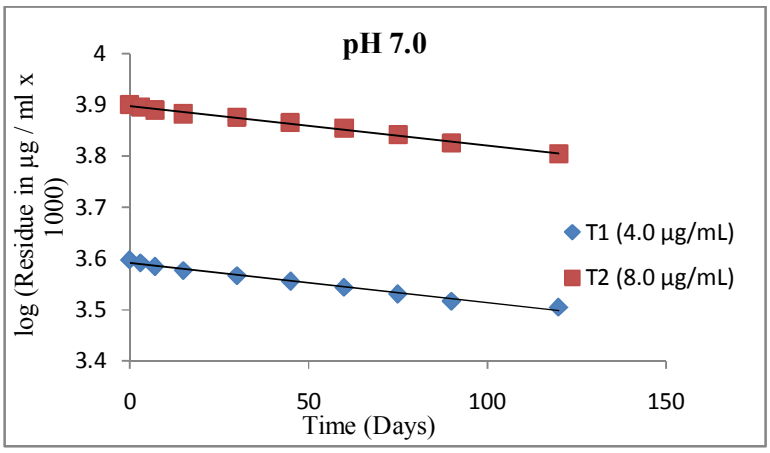

Figure 5.2: Dissipation of tebuconazole in water at pH 7.0

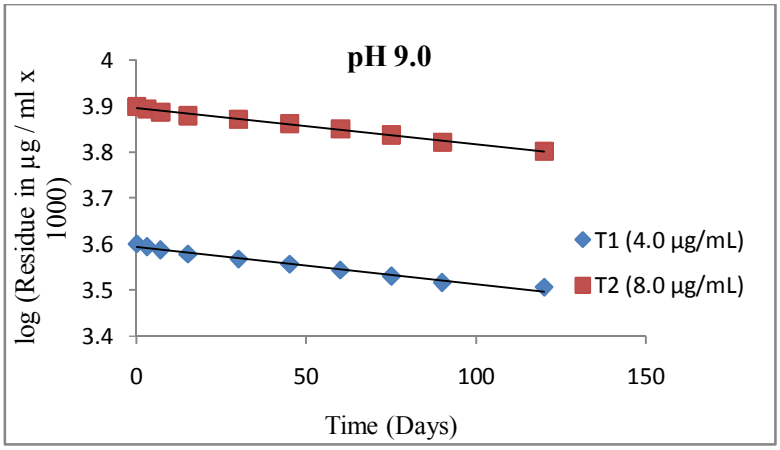

Figure 5.3: Dissipation of tebuconazole in water at pH 9.0

5.4 Reference

- EC, **1997**: EC C.7, **1997** "Degradation – Abiotic Degradation Hydrolysis as a Function of pH". The European Commission - Classification, Packaging and Labelling of Dangerous Substances in the European Union, Part 2 – Testing Methods.
- Frank R., Clegg B.S., Ripley B.D., Braun H.E., *Arch. Environ. Contam. Toxicol.* **1987**, *16*, 9.