

Photo Catalytic Applications Of Ferrous Doped Titanium Dioxide Nanoparticles In Dissipation Kinetics Of Tricyclazole Fungicide Residues In Different pH Waters Under Direct Sun Light

Tentu. Nageswara Rao¹, T. Benarji Patrudu*², M. V. Basaveswara Rao¹
Karri Apparao¹ and N. Krishna Rao¹

¹Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, India.

²Department of Chemistry, GITAM University, Hyderabad, Telangana, India.

Abstract: Studied the dissipation behavior of tricyclazole under direct sunlight using Fe doped TiO₂ nanoparticles (FeTiO₂) as catalyst. FeTiO₂ nanoparticles are synthesized and characterized by scanning electron microscopy (SEM) combined with energy Dispersive X-ray analysis (EDX) and Fourier transform Infrared Spectroscopy (FT-IR). The photocatalytic studies were conducted by the 1 litre of milli-Q water, pH 4.0, 7.0 and 9.0 buffer were spiked with 1 mL of 1000 mg/L stock solution of tricyclazole 75 % WP to get 1 µg/mL concentration of pesticide active in water. Three sets of such samples in triplicates were prepared and sonicated for 10 minutes to get homogeneous concentration of pesticide active in water and labeled as S1, S2, and S3. Two sets of samples (S1 and S2) were added FeTiO₂ nanoparticles to get 0.06g/L content of photocatalyst. S1 and S3 sets of samples were exposed to sun light. S2 set of samples were kept in an oven at 40°C in dark. The milli-Q water spiked with stock solution of pesticide and without addition of FeTiO₂ nanoparticles (S3) were also exposed to sun light for the measurement of photolytic degradation of pesticide in water. The FeTiO₂ added Milli-Q water without spiking of pesticides (S4) were also maintained as untreated control for the identification of absence of pesticide. The collected samples were quantified using a validated HPLC-UV method. Kinetic parameters such as rate constant (k), DT50 and DT90 were calculated using the dissipation data of tricyclazole.

Keywords: Fe doped TiO₂, Tricyclazole, HPLC-UV, SEM, TEM, DT50 and DT90.

I. Introduction

Tricyclazole (five-methyl-1, 2, four-triazolo [3,4-b]benzothiazole) is a completely unique fungicide for control of *Pyricularia oryzae* on rice. Tricyclazole is systemic in rice and could control rice blast disorder in any degree of plant improvement with the aid of a selection of software methods. Tricyclazole protects flowers from contamination by using *P. oryzae* by using preventing penetration of the epidermis with the aid of the fungus. The compound acts by way of inhibiting melanization inside the appressorium, as a result causing a loss of rigidity within the appressorial wall. Tricyclazole has no apparent effect on spore germination even though sporulation is reduced. Tricyclazole is not curative however is defensive in its activity. In the recent years the use of heterogeneous photo catalyst nano Fe doped TiO₂ (FeTiO₂) in the degradation and mineralization of herbicide, insecticide, N-heterocyclic compounds, saturated fatty acids, different organic dyes in water and gaseous pollutant in air using UV and visible-light has gained wide attention due to its low cost preparation, low toxicity, high stability and effectiveness than TiO₂ [5] When FeTiO₂ nanoparticles are subjected to UV, VIS or solar light, it gains energy from light and promote electrons (e-) from the valence band (VB) of TiO₂ to the conduction band (CB) leaving a positive hole (h+). Fe in FeTiO₂ trap electrons (e-) and positive holes (h+) from TiO₂ since the energy levels of Fe²⁺/Fe³⁺ lies close to that of Ti³⁺/Ti⁴⁺, and reduce the recombination of photo-generated electron and hole pair in TiO₂ and enhance the availability of electrons (e-) and positive holes (h+) in FeTiO₂. These electrons (e-) and positive holes (h+) are involving in the degradation of organic molecules by oxidation / reduction process [6, 7, 8]. Destroying of *Escherichia coli* bacteria in water have been achieved completely within an hour by deposited *Escherichia coli* bacteria in water on FeTiO₂ thin film and irradiating it in visible radiation [9, 10] based on the information present study was conducted to investigate the dissipation behavior of tricyclazole in three different buffers using FeTiO₂ as catalyst under natural climatic conditions in solar light.

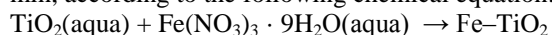
II. Materials And Methods

Reference analytical standard of tricyclazole (purity 97.5%), Titanium tetrachloride and Iron nitrate were obtained from Sigma Aldrich. The test item tricyclazole 75% Wettable powders (WP) was purchased from local market. Acetonitrile, Water HPLC grade, orthophosphoric acid Sodium hydroxide LR grade, Potassium chloride GR grade, Boric acid GR grade, Potassium biphthalate GR grade, Hydrochloric acid AR grade and

Potassium phosphate AR grade were obtained from the Merck India limited. Distilled water was purified by using the Milli-Q Plus apparatus (Millipore, Bedford, MA, USA).

2.1. Preparation of FeTiO₂ nanoparticles

TiO₂ nanoparticles were prepared by the drop wise addition of 5 mL of TiCl₄ (sigma Aldrich) in 100 mL distilled water containing 0.2 M HCl (AR Grade Purity- 36.6) at 5°C ± 0.5 °C and ultrasonicated for 1 hour at 82°C and kept for 18 hours at 82°C in a thermostat controlled oven (TiCl₄ + 2H₂O → TiO₂ + 4HCl). The obtained white precipitate was washed with distilled water ten times by using refrigerated centrifuge and finally washed with methanol. The methanol was then decanted and the precipitate (TiO₂ nanoparticles) was dried at 120°C for 4 hours. A 100 mL boiling solution of Iron nitrate (sigma Aldrich) was added drop wise to the boiling distilled water containing 2 g of TiO₂ nanoparticles. The solution was sonicated at 100°C about 30 min, according to the following chemical equation:



The obtained brown colour FeTiO₂ nanoparticles were washed with distilled water six times by using refrigerated centrifuge and finally washed with methanol. The FeTiO₂ nanoparticles dried at 120°C for 4 hours after decanted the methanol.

2.2. Standard stock solution

The stock solution of reference standard was prepared by weighing about 10 mg of tricyclazole of known purity into a 10 ml volumetric flask using an analytical balance having accuracy of 0.01 mg. The content of each flask were dissolved using HPLC grade acetonitrile and made up to the mark.

2.3. Sample stock solution

Accurately 132.77 mg of test item (purity 75.32%) of tricyclazole was taken into a 100 mL volumetric flask. The content was dissolved in 5 mL of acetonitrile, sonicated and made up to the mark with the acetonitrile. The concentration was 1000 mg/L solution. The stock sample solution was used for preparation of dose samples in different aqua's buffers.

2.4. Acidic Buffer

The buffer solution of pH 4.0 was prepared by dissolving 4.0 g of disodium hydrogen orthophosphate in 1.0 L milli-Q water and the pH was adjusted to 4.0 using 1.0 mole/L hydrochloric acid solution.

2.5. Neutral Buffer

The buffer solution of pH 7.0 was prepared by dissolving 4.0 g of potassium dihydrogen orthophosphate in 1.0 L milli-Q water and the pH was adjusted to 7.0 using 1.0 mole/L sodium hydroxide solution.

2.6. Basic Buffer

The buffer solution of pH 9.0 was prepared by dissolving 1.25 g of boric acid and in 1.0 L milli-Q water and the pH was adjusted to 7.0 using 1.0 mole/L sodium hydroxide solution.

2.7. Photo catalytic studies

The photocatalytic studies were carried out in a borosil glass bottle under sunlight at GITAM University, Hyderabad, To the one litre of milli-Q water, pH 4.0, 7.0 and 9.0 buffer were spiked with 1 mL of 1000 mg/L stock solution of pesticide formulation to get 1µg/mL concentration of pesticide active in water (each pesticide was spiked into separate one litre glass bottle). Three sets of such samples in triplicates were prepared and sonicated for 10 minutes to get homogeneous concentration of pesticide active in water and labelled as S1, S2, and S3. Two sets of samples (S1 and S2) were added FeTiO₂ nanoparticles to get 0.06g/L content of photocatalyst (optimum amount). The sample suspension of FeTiO₂ were sonicated in the dark for 10 min before exposure to the sunlight, to get even disperse of FeTiO₂ particles in water and attain adsorption equilibrium. S1 and S3 sets of samples were exposed to sun light from morning 8 am to evening 5 pm in the month of february. S2 set of samples were kept in an oven at 40°C in dark. The unexposed to sun light samples (S2) were maintained for the measurement of nonphotocatalytic degradation of pesticide active in water. Milli-Q water spiked with stock solution of pesticide and without addition of FeTiO₂ nanoparticles (S3) were also exposed to sun light for the measurement of photolytic degradation of pesticide in water. The FeTiO₂ added Milli-Q water without spiking of pesticides (S4) were also maintained as untreated control for the identification of absence of pesticides. The day temperature during the exposure period of soil samples under sunlight varied from 28 to 45°C. The intensity of the sunlight and temperature were measured during the exposure time using LUX meter.

2.8. Sampling data

Water samples were collected from the bottle at different depth on different occasion after exposure under sun light (0, 5, 9, 15, 18, 20, 27 and 36 hours for photocatalytic experiment. The collected water sample was centrifuged and filtered thoroughly 0.2 µ filter and analyzed in HPLC.

2.9. Chromatographic separation parameters

The HPLC-UV system used, consisted Shimadzu high performance liquid chromatography with LC-20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase Column

Phenomenex C18 (25 cm x 4.6 mm i.d X 5µm particle size.), oven temperature was maintained at 40°C. The injected sample volume was 20µL. Mobile Phases A and B was Acetonitrile and 0.1% orthophosphoric acid in HPLC water (75:25 (v/v)). The flow- rate used was kept at 0.7 mL/min with a detector wavelength at 235 nm. The external standard method of Calibration was used for this analysis.

2.10. Method validation

Method validation ensures analysis credibility. Recovery studies were conducted by fortifying three different concentrations of each fungicide at 0.03, 0.15 and 0.3 µg/g levels in four different buffers. Three replicates determinations were made at each concentration level along with two control. Based on the recovery study the limit of quantification was established. Linearity was determined by different known concentrations (0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 µg/mL) which were prepared by diluting the stock solution. The Limit of Detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control sample. The Limit of Quantification (LOQ, µg/mL) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

III. Results And Discussion

3.1. Description of FeTiO₂ nanoparticles

The scanning electron microscope (SEM) images of FeTiO₂ nanoparticles and Scanning electron microscopy (SEM) combined with energy dispersive X-ray analysis (EDX) for the quantitative determination and elemental composition of Fe/Ti ratio presented in **Fig. 1 and Fig. 2** indicates the Fe content was 0.5 % and Ti, O and Fe are the elemental compositions and the size of the particle was observed to be 20-24 nm. The Fourier transform Infrared Spectroscopy was showed peaks at (652–551 cm⁻¹), (1632 cm⁻¹) and (2235 cm⁻¹) indicated for molecular water, Ti-O and Ti-O-Fe stretching vibration band respectively and presented in **Fig. 3**.

3.2. Specificity

Specificity was confirmed by injecting the Mobile phase solvents i.e., Acetonitrile and 0.1% Orthophosphoric acid, HPLC water, sample solution standard solution and buffer controls (acidic, neutral, basic) There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues shown in **Fig. 4, Fig. 5 and Fig. 6**. Furthermore, the retention time of tricyclazole was constant at 4.3 ± 0.2 min.

3.3. Linearity

Different known concentrations of tricyclazole (0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 µg/mL) were prepared into a different 10 mL volumetric flasks by diluting the stock solution. These standard solutions were directly injected into a HPLC. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six standard concentration solutions. The details were presented in **Table 1**. The peak areas obtained from different concentrations of standards were used to calculate linear regression equation. This was $Y=15771X + 1.3059$ with correlation coefficient of 0.9998 respectively. A calibration curve is showed in **Fig. 7**.

3.4. Recovery

The methods were observed in precision with a acceptable range < 20 % of RSD when injected 10 x LOQ recovery sample five times consecutively into the HPLC. The statistical parameters such as standard deviation (SD) and percentage of relative standard deviation (% RSD) were presented in **Table 2**. The method had an acceptable recovery range (70 - 110 %) for fungicide in four different soil. The Limit of Quantification (LOQ) was established as 0.03 mg/L from 10:1 peak to noise height ratio. The statistical parameters for recovery such as mean recovery percentage, standard deviation (SD), percentage of relative standard deviation (% RSD) and Horwitz Limit are presented in **Table 2**. The formula for calculation residue and statistical parameters are presented below the equivation

$$\text{Residue content } (\mu\text{g/mL}) = \frac{A \times C}{D}$$

where,

- A - Peak area of active content in sample (µV*sec)
 - C - Concentration of the standard solution µg/mL)
 - D - Peak area of active content in standarde solution (µV*sec)
- Recovered residue x 100

Recovery % =

$$\frac{\text{Fortified concentration}}{\text{Standard deviation} \times 100}$$

% RSD =
Mean
Horwitz Limit = $2^{-(1-0.5 \times \log C)} \times 0.67$
where,
C – Concentration

3.5. Photo catalytic decontamination of pesticide in water

On 0 hour analysis of tricyclazole fortified water showed that the residue of tricyclazole as 1.01 µg/L, 1.03 µg/L, 0.99 µg/L, and 1.02 µg/L, for milli-Q water, pH 4, pH 7 and pH 9 buffer water with 0.06 g/L load of catalyst respectively. The residues of tricyclazole dissipated to 0.33 µg/L, 0.31 µg/L on 18th h for milli-Q water, pH 7, for pH 4 and pH 9 dissipated to 0.21 µg/L, 0.33 µg/L on 9th h. On 27th h the tricyclazole concentration degraded to near the LOQ level for milli-Q water and pH 7, and got complete degradation on 36th h but pH 4 and pH 7 buffer water got complete degradation on 20th h.

The summarized results for photocatalytic studies are presented in **Table 3 and Figure 8**. The data clearly demonstrate that the decontamination of pesticide follows pseudo-first-order kinetics in FeTiO₂ loaded water (S1) when calculated residues values with time by using below the first order kinetic formula (OECD 111). The absence of pesticide residues in S4 were observed because of no pesticide were applied and no degradation of fungicide in S2 were observed due to inactivation of the FeTiO₂ in the absence of light. DT50 and DT90 values were calculated using the following formulas

$$DT50 = \ln 2 / (k) \text{ and } DT90 = \ln 10 / (k)$$

Where,

'k' is slope of the curve obtained from the dissipation data.

The calculated DT50 and DT90 values are presented in **Table 4**. The rate constant value was calculated by linear regression equation from the first order rate equation.

$$K = \ln a/a-x/dt$$

Where, dt is the time interval between t1 and t2 and a, x are the concentration of pesticides at times t1 and t2 respectively. A plot of concentration of the residues and rate with the R² indicates first order kinetics in dissipation of fungicide. The, DT90 of tricyclazole calculated by regression analysis from the dissipation data. Results clearly indicate that the rate constant was high when the FeTiO₂ was present in water than in absence of water and no degradation of pesticide in S2 set of water which were kept in dark. The decontamination was fast when studied under sunlight in presence of FeTiO₂ in water due to the formation of electrons (e⁻) and positive hole (h⁺) in TiO₂ when it absorbed energy from sun light and the availability of electrons (e⁻) and the positive holes (h⁺) pairs which were contributing the simultaneous oxidation and reduction of pesticide in soil were enhanced by Fe in FeTiO₂. This was confirmed by the no degradation of pesticide in water samples when store in dark. Absence of pesticide residues were also observed in water spiked (S4) water samples because of no pesticide was applied

IV. Conclusion

The FeTiO₂ nanoparticles were observed to be excellent decontaminating catalyst for tricyclazole in different water samples. In the absence of catalyst the compound persists several days. The mobile phase Acetonitrile and 0.1% orthophosphoric acid in HPLC water showed good separation and resolution and the analysis time required for the chromatographic determination of three different type of buffers is very short (around 15 min for a chromatographic run).

Satisfactory validation parameters such as linearity, recovery, precision and LOQ and DT 50 values were established by following South African National Civic Organization (SANCO) and Environmental Protection Agency (EPA) guidelines. Therefore, the proposed analytical procedure and dissipation data could be useful for regular monitoring, residue labs and research scholars to determine the tricyclazole residues in different commodities (crop, water and soil samples).

Acknowledgement

The authors are thankful to the Dr. K Raghu Babu, Professor, Department of Engineering Chemistry, Andhra University, Visakhapatnam for providing necessary facility to conduct the Laboratory experiment.

References

- [1]. Yasuko SHIBA, Toshimi NAGATA, The Mode of Action of Tricyclazole in Controlling Rice Blast. *Japanese Journal of Phytopathology* 47, 1981, 662-667.
- [1] Kunova A, Pizzatti C, Cortesi P, Impact of tricyclazole and azoxystrobin on growth, sporulation and secondary infection of the rice blast fungus, *Magnaporthe oryzae*. *Pest Manag Sci* 69(2), 2012, 78-84

- [2] ANIRUDHA CHATTOPADHYAY, CHANDA KUSHWAHA, R. CHAND and J.S, SRIVASTAVA, Differential mode of action of tricyclazole in vitro and in planta on Bipolaris sorokiniana causing spot blotch in barley. *Indian Phytopathology* 66(2), 2013, 155-158.
- [3] Balcer JL, DeAmicis CV, Johnson PL, Klosin J, Whiteker GT, Rao CS, Dai D, Synthesis of new compounds related to the commercial fungicide tricyclazole. *Pest Manag Sci.* 67(5) 2011,556-9.
- [4] Dang T. T. Nhung, Thai K. Phong, and Hirozumi Watanabe, Determination of Tricyclazole in Water Using Solid Phase Extraction and Liquid Chromatography. *Journal of Liquid Chromatography & Related Technologies* 32, 2009, 2712–2720.
- [5] Xiao YX1, Fan DF, Chen HX, Gas chromatographic determination of systemic fungicide tricyclazole in soil and water. *J Assoc Off Anal Chem* 73(5), 1990, 761-3.
- [6] Sun H, BaiY, ChengY, JinW, Xu N, Preparation and Characterization of Visible- Light- Driven Carbon-Sulfurcodoped TiO₂ Photocatalysts. *Industrial and Engineering Chemistry Research* 45, 2006, 4971-4976.
- [7] Tanmay K, Ghora, Synthesis of spherical Mesoporous Titania Modified Iron-Niobate Nanoclusters for Photocatalytic Reduction of 4-Nitrophenol. *Journal of materials research and technology* 4(2), 2015, 133-143.
- [8] Yana Ruzmanova, Marco Stoller, Angelo Chianese, Photocatalytic Treatment of Olive Mill Waste Water by Magnetic Core Titanium Dioxide Nanoparticles. *The Italian Association of Chemical Engineering.* 32, 2013, 2269-2274.
- [9] Zhang Q, GaoL, J Guo, Effects of Calcination on the Photocatalytic Properties of Nanosized TiO₂ Powders Prepared by TiCl₄ Hydrolysis. *Applied Catalysis B* 26, 2000, 207-215.

Fig. 1. EDX analysis of FeTiO₂ nanoparticles

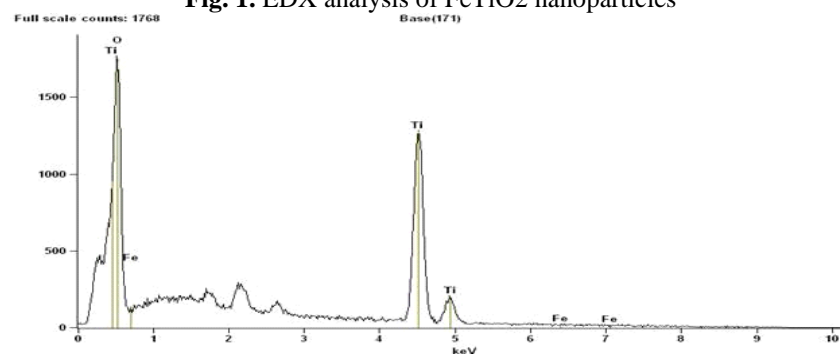


Fig. 2. SEM Image of FeTiO₂ nanoparticles

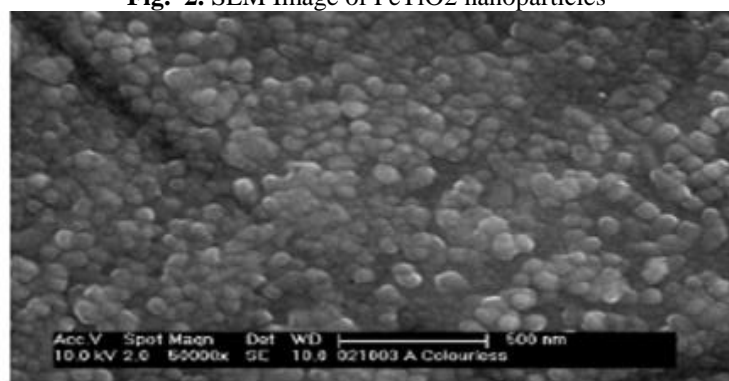


Fig. 3. FT-IR Spectra of FeTiO₂ nanoparticles

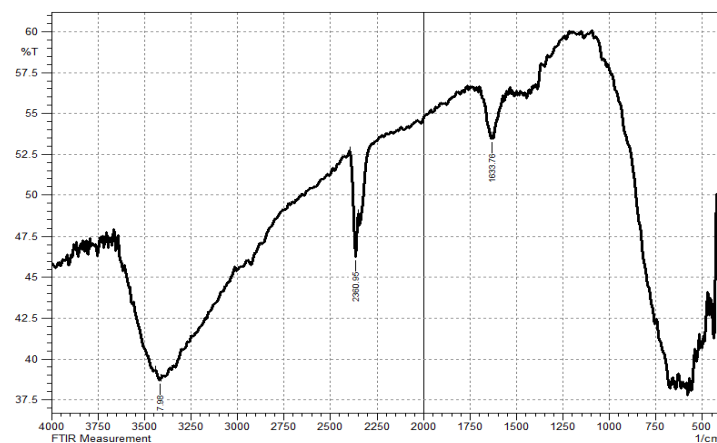


Fig.4. Representative Chromatogram of tricyclazole test item in acidic water- 5th hour

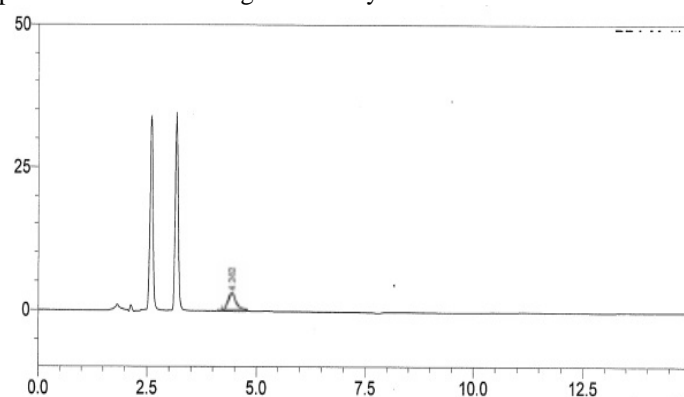


Fig.5. Representative Chromatogram of tricyclazole test item in neutral water -9th hour

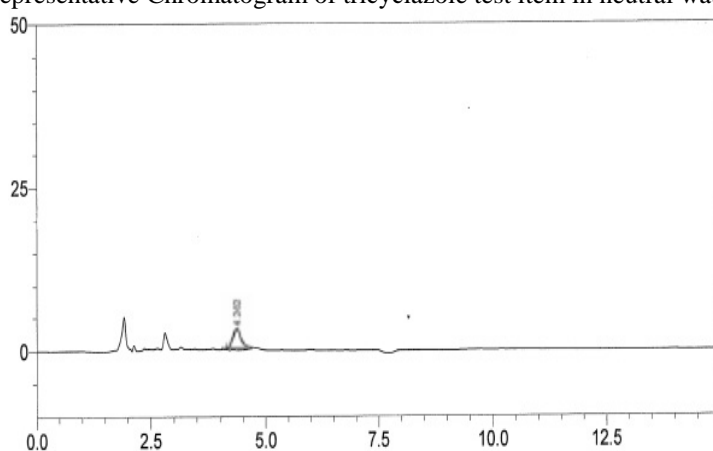


Fig.6. Representative Chromatogram of tricyclazole test item in basic water - 5th hour

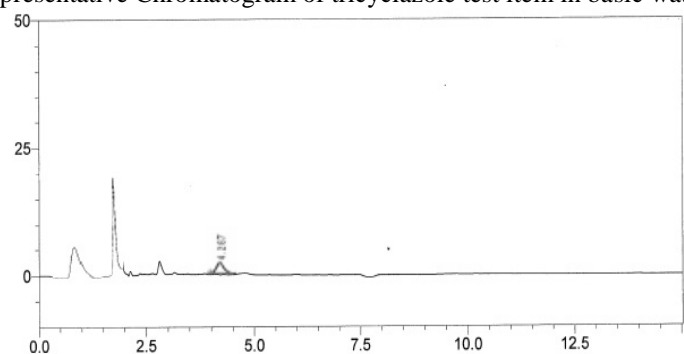


Fig.7. Representative Calibration curve of tricyclazole standard

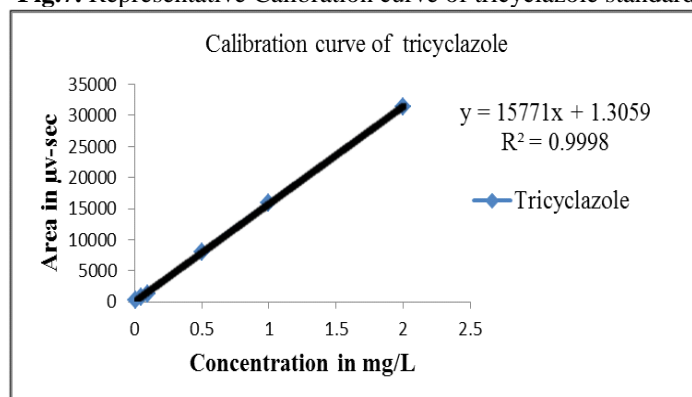


Fig. 8. Graph representing the dissipation curve of photocatalytic decontamination of tricyclazole in water under direct sunlight

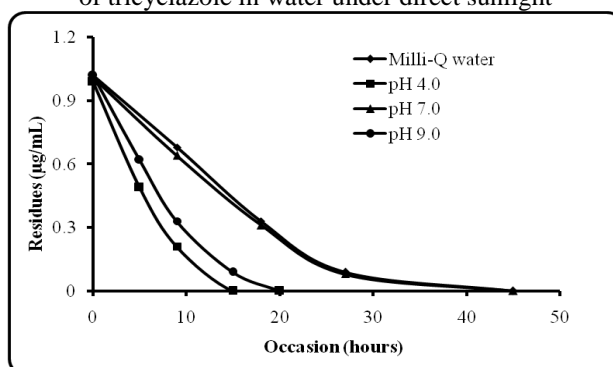


Table 1. Calibration details – tricyclazole

Concentration in (mg/L)	Peak area of tricyclazole ($\mu\text{v}\text{-sec}$)
2	31425
1	15964
0.5	8029
0.1	1337
0.05	779
0.01	197

Table 2. Precision details – tricyclazole

Statistical Parameters	Compound Name Tricyclazole
Precision	
Mean	99
SD	1.25
% RSD	1.33
Recovery	
Mean	99
SD	1.29

Table 3. Dissipation data for photocatalytic decontamination of tricyclazole in water under direct sunlight

Tricyclazole					
Occasion (hours)	Residues ($\mu\text{g}/\text{mL}$)		Occasion (hours)	Residues ($\mu\text{g}/\text{mL}$)	
	Milli-Q Water	pH 7.0		pH 4.0	pH 9.0
0	1.01	1.03	0	0.99	1.02
9	0.68	0.64	5	0.49	0.62
18	0.33	0.31	9	0.21	0.33
27	0.09	0.08	15	BDL	0.09
36	BDL	BDL	20	BDL	BDL

Table 4. Kinetic parameters for photocatalytic decontamination of tricyclazole in water under direct sunlight

Kinetic parameters	Water			
	Milli-Q Water	pH 4.0	pH 7.0	pH 9.0
Tricyclazole				
DT_{50} (h)	7.79	3.97	7.46	4.26
DT_{90} (h)	25.88	13.20	24.78	14.14
k ($1 \times 10^{-3} \text{ h}^{-1}$)	89.0	174.4	92.9	162.9