ISSN : 0974-7419 Volume 16 Issue 10

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 16(10) 2016 [407-416]

Dissipation kinetics of copper oxychloride and kasugamycin fungicide residues in different pH waters

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ABSTRACT

To study the dissipation behaviour of Copper oxychloride and Kasugamycin in acidic, neutral and basic water of pH 4.0, 7.0 and 9.0, a laboratory experiment was conducted by the Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh. Required quantity of Copper Oxychloride 45% + Kasugamycin 5% WP formulation, was spiked in pH 4.0, 7.0 and 9.0 aqueous buffer solutions to give uniform concentrations of T0 – Untreated Control, T1 – Copper Oxychloride 45% + Kasugamycin 5% WP @ 1.0 µg/mL and T2 – Copper Oxychloride 45% + Kasugamycin 5% WP @ 2.0 µg /mL. The sampling occasions are 0, 1, 3, 5 and 7 days for Copper oxychloride and 0, 6, 12, 24 and 48th hour for Kasugamycin. Samples collected on different occasions were analysed for Copper oxychloride and Kasugamycin content. Samples were analysed until the residues are below detectable level. All the samples were processed and analysed for the residues by a validated pectrophotometric method for Copper oxychloride and Kasugamycin was analysed by a validated HPLC-UV method. The DT_{50} (Half Life) of Copper oxychloride and Kasugamycin calculated by regression analysis from the dissipation data.

KEYWORDS

Copper oxychloride 45% + kasugamycin 5% WP formulation;
DT50;
Residues and aqueous buffer solutions.

INTRODUCTION

Fungicides are the essential part of agriculture crop management for better yields. In this process several new molecules have been introduced for the potential control of pests and diseases. Fungicides can be divided into protectant and specific types[1]. Protectants are the older type and includes copper and sulfur based products. They form a protective film on the plant surface and inhibit the germination of fungal spores[1] Specific type fungicides are so called because they act on one specific chemical reaction in the fungus. Dicopper chloride trihydroxide is the chemical compound with the formula $Cu_2(OH)_3Cl$. It is often referred to as tribasic copper chloride[2]. It is a greenish crystalline solid encountered in mineral deposits, metal corrosion products, industrial products, art and archeological objects and some living systems. Copper oxychloride is a protectant fungicide/bactericide which prevents infection on plants. Its mode of action is by interfering with the enzyme system of spores and mycelium, a process which is usually irreversible. It forms a chemical barrier against fungal attack.

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Kasugamycin is an aminoglycoside antibiotic that was originally isolated in 1965, from steptomyces kasugaensis, a streptomyces strain found near the kasuga shrine in nara, japan. Kasugamycin was discovered by hamao umezawa, who also discovered kanamycin and bleomycin, as a drug which could prevent growth of fungus causing rice blast disease[3]. It was later found to inhibit bacterial growth also. It exists a white, crystalline substance with the chemical formula C14H28ClN3O10. It is also known as kasumin.

MATERIALS AND METHODS

Reference analytical standards of Copper Oxychloride (Purity 95%) and Kasugamycin (Purity 99.4%) were obtained from Sigma Aldrich. The test item Copper Oxychloride 45% + Kasugamycin 5% Wettable Powder (WP) was purchased from local market. Acetonitrile, Water HPLC grade, 1-Heptane sulphuric acid sodium salt HPLC grade, Ortho Phosphoric acid AR grade, Citric acid LR grade, EDTA salt GR grade, Sodium diethyl dithio-carbamate GR grade, Butyl acetate GR grade and Concentrated sulphuric acid GR grade were obtained from the Merck India limited. Distilled water was purified by using the Milli-Q Plus apparatus (Millipore, Bedford, MA, USA). Shimadzu High Performance Liquid Chromatography system equipped with LC-20 ATvp pump and SPD-20A UV/VIS CTO-20A Column oven using LC soluition software, Shimadzu UV-1601, UV-VIS spectrophotometer supplied by M/s. Shimadzu corporation, Kyoto, Japan, Hamilton syringe (25µl) – M/s. Hamilton Inc., New York, USA, Volumetric flasks, pipettes, measuring cylinder and glass columns - All 'A' grade glassware supplied by M/s. Borosil Glass and Glassware Mumbai, India and Mettler AG-245 analytical balance, capable of weighing 0.01 mg supplied by M/s. Mettler Toledo, Switzerland.

Chromatographic separation parameters for kasugamycin

Instrument - Shimadzu High Performance Liquid Chromatograph system equipped with LC-20 ATvp pump and SPD-20A UV/VIS CTO-20A Column oven using LC soluition software.
Column used - Phenomenex C_{18} (25cm length x 4.6mm i.d)
Wave length - 235 nm
Mobile phase - Acetonitrile : Water : 1-Heptane sulphonic acid sodium salt (100:900 : 0.1 v/v/w) Adjust pH to 3.5 with diluted phosphoric acid
Flow rate - 1.0 ml/min
Injected volume - 10 µl
Retention time
Kasugamycin - About 6.0 minutes

Spectrophotometric separation parameters for Copper Oxychloride

Instrument - Shimadzu spectrophotometer UV 1700
Cuvette - 1cm^2 quartz cell
Measuring mode - Absorbance
Absorbance measured at fixed wavelength (λ max) - 435 nm

METHOD VALIDATION

Preparation of stock solution of reference analytical standard of copper oxychloride

Accurately weighed 50.16 mg of reference analytical standard of Copper oxychloride in 50 ml volumetric flask and the volume was made upto the mark using double distilled water.

Preparation of stock solution of reference analytical standard of kasugamycin

Accurately weighed 10.31 mg of reference analytical standard of Kasugamycin in 10ml volumetric flask and the volume was made upto the mark using acetonitrile.

Preparation of calibration solutions of copper oxychloride

Different known concentrations of Copper oxychloride analytical standard solutions were prepared in double distilled water to contain copper @ 0.03, 0.1, 0.5, 1, 2 and 5 µg/ml and the absorbance was measured at 435 nm after forming the complex as

TABLE 1 : Calibration details – copper oxychloride

Copper Concentration(mg/L)	Absorbance at 435nm
0.03	0.013
0.1	0.035
0.5	0.135
1	0.287
2	0.574
5	1.402

TABLE 2 : Calibration details – kasugamycin

Injected concentration (mg/L)	Response in Area (µV-sec)
0.01	235
0.1	2793
0.5	10124
1	22397
2	43925
5	109984

described in methodology 5.2 (Complex formation). A calibration curve was constructed between absorbance versus concentration and the curve was found linear. The details were presented in TABLE 1 followed by calibration curve (Figure 1).

Preparation of calibration solutions of kasugamycin

Different known concentrations of Kasugamycin (0.01-5 mg/L) were prepared in mobile phase by diluting the stock solution. Injected the standard solutions and measured the peak area. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of the method was determined from the correlation coefficient[4,5,6]. The details were presented in TABLE- 2. Calibration curve was shown in Figure 2.

Recovery – limit of determination

Recovery studies in acidic water, neutral water and basic water was conducted by fortifying different concentrations of mixture of Copper oxychloride (0.1 – 1.0 mg/L) and Kasugamycin (0.05 – 0.5 mg/L). The samples were homogenized, extracted and analysed for Copper oxychloride and Kasugamycin content, as described in the method of analysis.

The average percent recovery for Copper oxychloride was 88 ± 1.00, 87 ± 1.15 and 86 ± 1.53 at 0.1 mg/L and 93 ± 1.53, 93 ± 2.00 and 90 ± 2.65 at 1.0 mg/L fortification levels for acidic, neutral and basic water respectively.

The average percent recovery for Kasugamycin was 87 ± 1.15, 87 ± 1.15 and 85 ± 1.15 at 0.05 mg/L and 94 ± 2.05, 93 ± 2.61 and 91 ± 1.01 at 0.5 mg/L fortification levels for acidic, neutral and basic water respectively.

The method has a limit of determination 0.1 mg/L (LOQ) for Copper oxychloride and 0.05 mg/L (LOQ) for Kasugamycin.

Method of analysis of Kasugamycin and Copper oxychloride

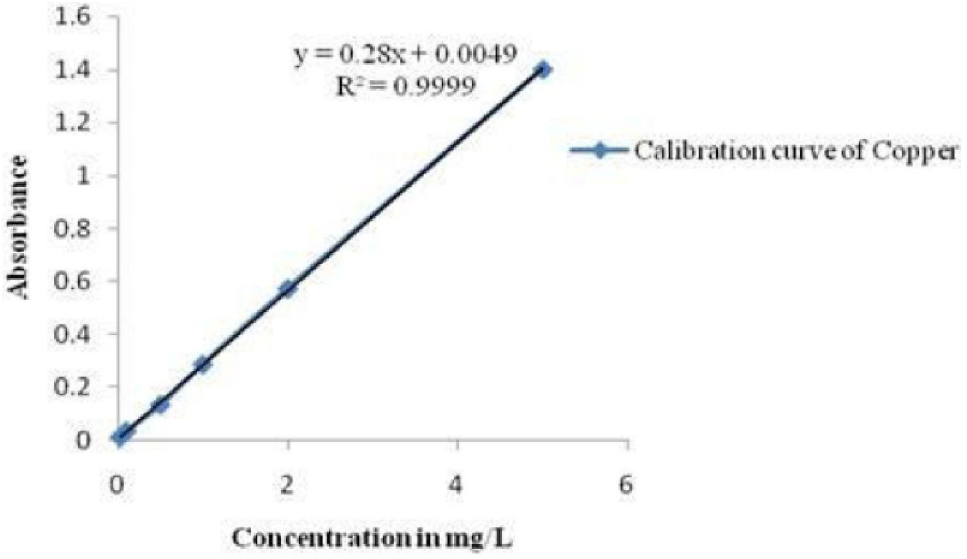

Figure 1 : Representative calibration curve of copper oxychloride standard

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The representative sample was filtered in Whatman 0.45 micron filter paper and injected the samples directly into HPLC.

Determination of copper content

Complex formation

Exactly 25 ml of representative water sample was pipetted out into a beaker, added 5.0 mL of 25% aqueous citric acid solution, render slightly alkaline with dilute ammonia solution and boil off the excess of ammonia. Alternatively, adjusted to pH 8.5 using a pH meter. Added 15.0 mL of 4% EDTA solution and cool to room temperature. Transferred to a separatory funnel, added 10 mL of 0.2% aqueous sodium diethyl dithio carbamate solution, and shake for 1 minute. A yellow brown colour develops in the solution. Pipette 20 mL of butyl acetate into the funnel and shake for 30 seconds. The organic layer acquires a yellow colour. Cool shake for 15 seconds and allow the phases to separate. Remove the lower aqueous layer added 20 mL of 5% sulphuric acid (v/v), shake for 15 seconds cool, and separate the organic phase. Determined the absorbance at 435 in 1 cm absorption cells against a blank.

Method of calculation

Copper content X (µg/ml) = y = mx+c from the calibration curve

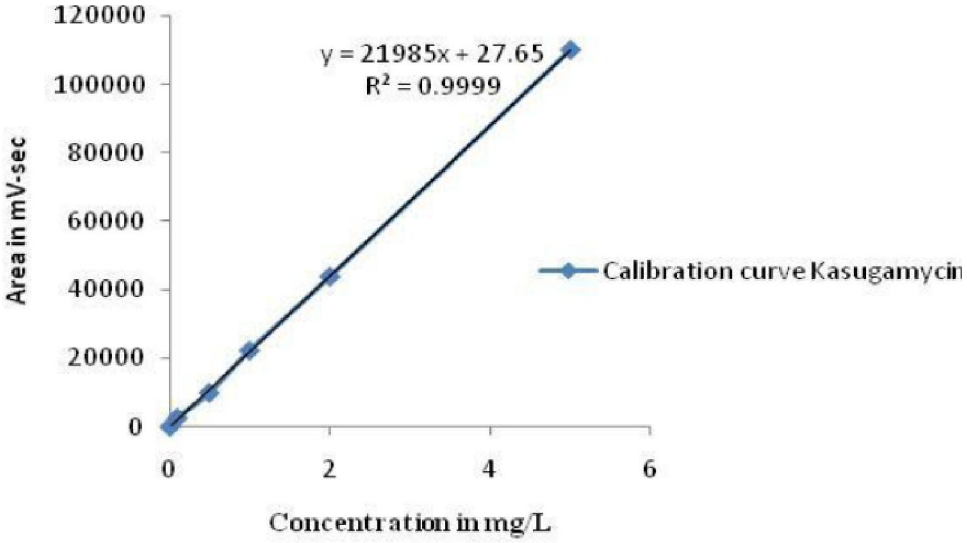

Figure 2 : Representative calibration curve of kasugamycin standard

TABLE 3 : Dissipation study in acidic water

Type of water		Acidic water (pH 4.0)
Location		SVV University, Department of Analytical Chemistry
Storage condition		Minimum: 22.7°C; Maximum: 29.3°C
Application data		
Preparation of Acidic water	pH 4.0	About 8 g of potassium dihydrogen phosphate will be dissolved in water and diluted to 1000 ml. The pH will be adjusted to 4.0 with the same.
Replications		Three
Method of fortification		Required quantity of the test item was fortified in water samples to get the uniform concentrations. T1: @ 1.0 µgml and T2: @ 2.0 µg /ml
Dose rate		T0 – Untreated Control T1 – Kasugamycin + Copper oxychloride 50 WP @ 1.0 µg/ml T2 – Kasugamycin 50% + Copper oxychloride 50 WP @ 2.0 µg/ml

$$x = \frac{y-c}{m}$$

Where; y – Absorbance; m – Slope; c – Intercept; X - Copper content in ppm

$$\text{Copper oxychloride content } (\mu g/ml) = \frac{X}{W} \times 25 \times 1.68 \times 1$$

where, X - Copper content (µg/ml); W - Weight of the sample (g); 25 - Sample extract volume (ml); 1.68 - Conversion factor; 1 - Dilution factor

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

Where; A - Peak area of the sample (µV-sec); C - Concentration of the standard (ppm); D - Peak area of the standard (µV-sec)

Experimental design

TABLE 4 : Dissipation study in neutral water

Type of water	Neutral water (pH 7.0)	
Location	SVV University, Department of Analytical Chemistry	
Storage condition	Minimum	Maximum
	22.7°C	29.3°C
	Application data	
Preparation of Acidic water	pH 7.0	About 8 g of potassium dihydrogen phosphate will be dissolved in water and diluted to 1000 ml. The pH will be adjusted to 4.0 with the same.
Replications	Three	
Method of fortification	Required quantity of the test item was fortified in water samples to get the uniform concentrations. T1: @ 1.0 µgml and T2: @ 2.0 µg /ml	
Dose rate	T0 – Untreated Control T1 – Kasugamycin + Copper oxychloride 50 WP @ 1.0 µg/ml T2 – Kasugamycin 50% + Copper oxychloride 50 WP @ 2.0 µg/ml	

TABLE 5 : Dissipation study in basic water

Type of water	Neutral water (pH 9.0)	
Location	SVV University, Department of Analytical Chemistry	
Storage condition	Minimum	Maximum
	22.7°C	29.3°C
	Application data	
Preparation of Acidic water	pH 9.0	About 12.5 g of boric acid and 15g of potassium chloride will be dissolved in water and diluted to 1 litre. The pH will be adjusted to 9.0 using sodium hydroxide.
Replications	Three	
Method of fortification	Required quantity of the test item was fortified in water samples to get the uniform concentrations. T1: @ 1.0 µgml and T2: @ 2.0 µg /ml	
Dose rate	T0 – Untreated Control T1 – Kasugamycin + Copper oxychloride 50 WP @ 1.0 µg/ml T2 – Kasugamycin 50% + Copper oxychloride 50 WP @ 2.0 µg/ml	

TABLE 6 : Regression analysis – acidic water for of copper oxychloride

Parameters	Dosages	
	T1	T2
Regression equation	Y = -0.26767 – 0.02365 * X	Y = -0.0080386 – 0.023431* X
Half-life (Hours)	12.73	12.85
Correlation co-efficient	-0.994	-0.993

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RESULTS AND DISCUSSION

Linearity

The method was found to be linear with a Correlation Coefficient of 0.9999 when tested in the range (0.03 – 5 mg/L) for Copper oxychloride. Kasugamycin over the linear concentration range (0.01 – 5 mg/L) the correlation coefficient was found to be 0.9999.

Recovery

Recovery studies in acidic water, neutral water and basic water was conducted by fortifying different concentrations of mixture of Copper oxychloride (0.1 – 1.0 mg/L) and Kasugamycin (0.05 – 0.5 mg/L). The samples were homogenized, extracted and analysed for Copper oxychloride and Kasugamycin content, as described in the method of analysis.

The average percent recovery for Copper oxychloride was 88 ± 1.00, 87 ± 1.15 and 86 ± 1.53 at 0.1 mg/L and 93 ± 1.53, 93 ± 2.00 and 90 ± 2.65 at 1.0 mg/L fortification levels for acidic, neutral and basic water respectively[7,8,9]. The average percent recovery for Kasugamycin was 87 ± 1.15, 87 ± 1.15 and 85 ± 1.15 at 0.05 mg/L and 94 ± 2.05, 93 ± 2.61 and 91 ± 1.01 at 0.5 mg/L fortification levels for acidic, neutral and basic water respectively. The method has a limit of determination 0.1 mg/L (LOQ) for Copper oxychloride and 0.05 mg/L (LOQ) for Kasugamycin.

Persistence details

Acidic water

Copper oxychloride

Analysis of acidic water samples on 0 day showed copper oxychloride residues 0.9970 mg/L and 1.9776 mg/L in tested dosages T1 and T2. By 1st day 0.9398 mg/L and 1.8148 mg/L. On 3rd day the residues were 0.7128 mg/L and 1.1846mg/L. At 5th day the residues were 0.2078mg/L and 0.4310mg/L in T1 and T2 dosages. By 7th day the residues of copper oxychloride dissipated to below the limit of determination in both the tested dosages.

Kasugamycin

The initial concentration of Kasugamycin at 0 hour in T1 and T2 tested dosages of acidic buffer was 0.509 mg/L and 0.937 mg/L which on 6th hour had dissipated to 0.382 mg/L and 0.693 mg/L respectively. On 12th hour, the levels of Kasugamycin were 0.325 and 0.585 mg/L and on 24th hour, the levels were 0.136 mg/L and 0.254 mg/L. Analysis of 48th hour samples showed that the residues were below the limit of determination in both the tested dosages.

Neutral water

Copper oxychloride

Analysis of neutral water samples on 0 day showed copper oxychloride residues 0.9902 mg/L and 1.9890 mg/L in tested dosages T1 and T2. By 1st day 0.9224 mg/L and 1.8066 mg/L. On 3rd day the residues were 0.7344 mg/L and 1.3506 mg/L. At 5th day the residues were 0.1900mg/L and 0.4022mg/L

TABLE 7 : Regression analysis – acidic water for of kasugamycin

Parameters	Dosages	
	T1	T2
Regression equation	Y = 0.0831– 0.1322 * X	Y = 0.3617 – 0.1315* X
Half-life (Days)	2.28	2.29
Correlation co-efficient	-0.9208	-0.9607

TABLE 8 : Regression analysis – neutral water for of copper oxychloride

Parameters	Dosages	
	T1	T2
Regression equation	Y = 0. 0861– 0.1377* X	Y = 0.3751 – 0.1344 * X
Half-life (Days)	2.19	2.24
Correlation co-efficient	-0.9076	-0.9310

TABLE 9 : Regression analysis – neutral water for of kasugamycin

Parameters	Dosages	
	T1	T2
Regression equation	Y = -0.27051 – 0.02406 * X	Y = 0.0069624 – 0.0238612* X
Half-life (Hours)	12.51	12.62
Correlation co-efficient	-0.993	-0.997

TABLE 10 : Regression analysis – basic water for of copper oxychloride

Parameters	Dosages	
	T1	T2
Regression equation	Y = 0.0938 – 0.1621* X	Y = 0.3884– 0.1512 * X
Half-life (Days)	1.86	1.99
Correlation co-efficient	-0.9143	-0.9306

TABLE 11 : Regression analysis – basic water for of kasugamycin

Parameters	Dosages	
	T1	T2
Regression equation	Y = -0.28683 – 0.02684 * X	Y = -0.01008 – 0.02613 * X
Half-life (Hours)	11.22	11.52
Correlation co-efficient	-0.995	-0.993

in T1 and T2 dosages. By 7th day the residues of copper oxychloride dissipated to below the limit of determination in both the tested dosages.

Kasugamycin

The initial concentration of Kasugamycin at 0 hour in T1 and T2 tested dosages of neutral buffer was 0.505 mg/L and 0.953 mg/L which on 6th hour had dissipated to 0.375 mg/L and 0.730 mg/L respectively. On 12th hour, the levels of Kasugamycin were 0.324 and 0.598 mg/L and on 24th hour, the levels were 0.132 mg/L and 0.255 mg/L. Analysis of 48th hour samples showed that the residues were below the limit of determination in both the tested dosages.

Basic water

Copper oxychloride

Analysis of basic water samples on 0 day showed copper oxychloride residues 0.9942 mg/L and 1.9894 mg/L in tested dosages T1 and T2. By 1st day 0.8750 mg/L and 1.8238 mg/L. On 3rd day the residues were 0.6732 mg/L and 1.2934 mg/L. At 5th day the residues were 0.1410 mg/L and 0.3324 mg/L in T1 and T2 dosages. By 7th day the residues of copper oxychloride dissipated to below the limit of determination in both the tested dosages.

Kasugamycin

The initial concentration of Kasugamycin at 0 hour in T1 and T2 tested dosages of basic buffer was 0.479 mg/L and 0.906 mg/L which on 6th hour had dissipated to 0.378 mg/L and 0.733 mg/L respectively. On 12th hour, the levels of Kasugamycin were 0.263 and 0.494 mg/L and on 24th hour, the levels were 0.112 mg/L and 0.222 mg/L. Analysis of 48th hour samples showed that the residues were below the limit of determination in both the tested dosages.

The dissipation curve plotted between concentration of the analyte and sampling occasions is presented in Figure 3, Figure 4, Figure 5, Figure 6, Figure 7 and Figure 8. DT50 value (Jyot, Gagan et al. 2010 ; Vijay Tularam Gajbhive et al. 2011) was calculated using the following formula

DT50 = ln 2/ (k)

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

The calculated DT 50 (Time required to degrade 50% of residues) values are presented in TABLE 6, 7 and 8. The rate constant value was calculated by

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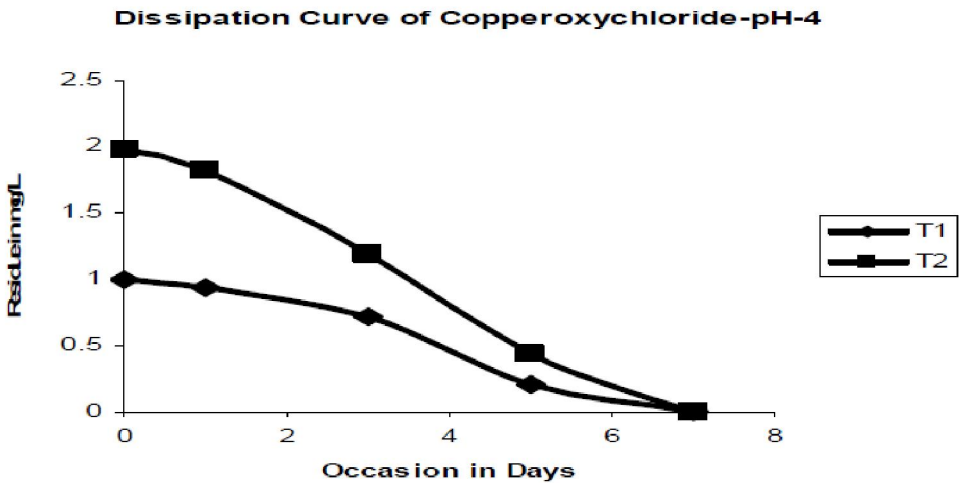

Figure 3 : Dissipation curve of copper oxychloride in acidic water

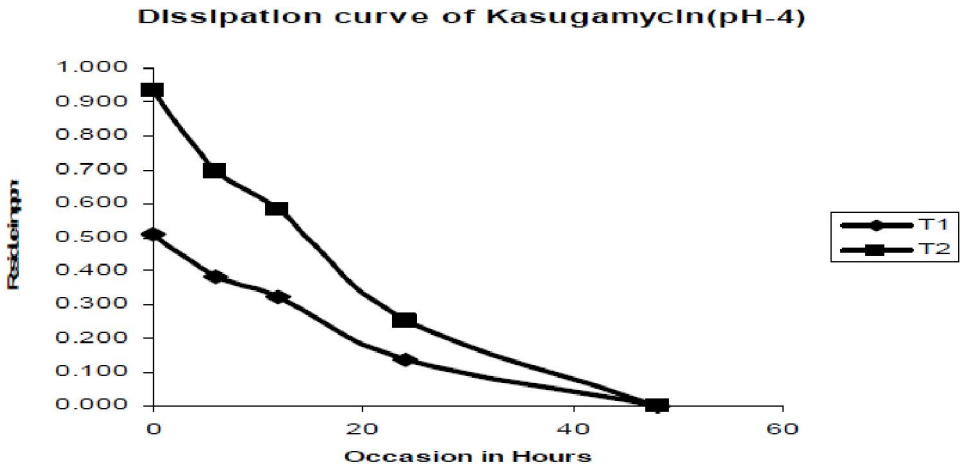

Figure 4 : Dissipation curve of kasugamycin in acidic water

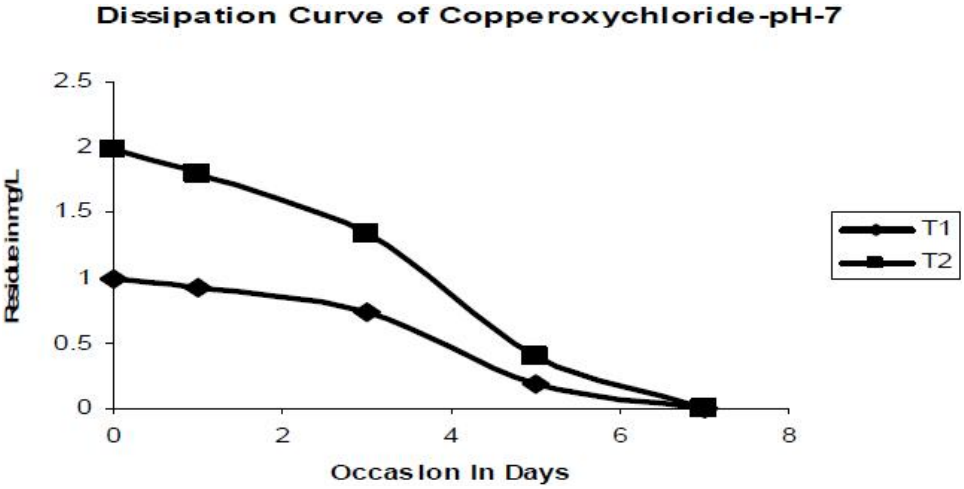

Figure 5 : Dissipation curve of copper oxychloride in neutral water

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 both the fungicides[10,11,12].

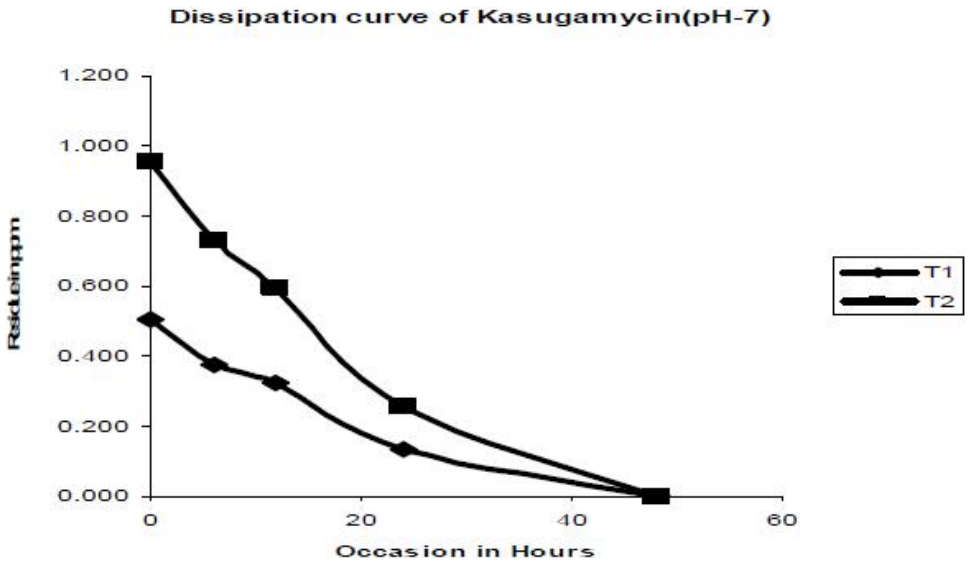

Figure 6 : Dissipation curve of kasugamycin in neutral water

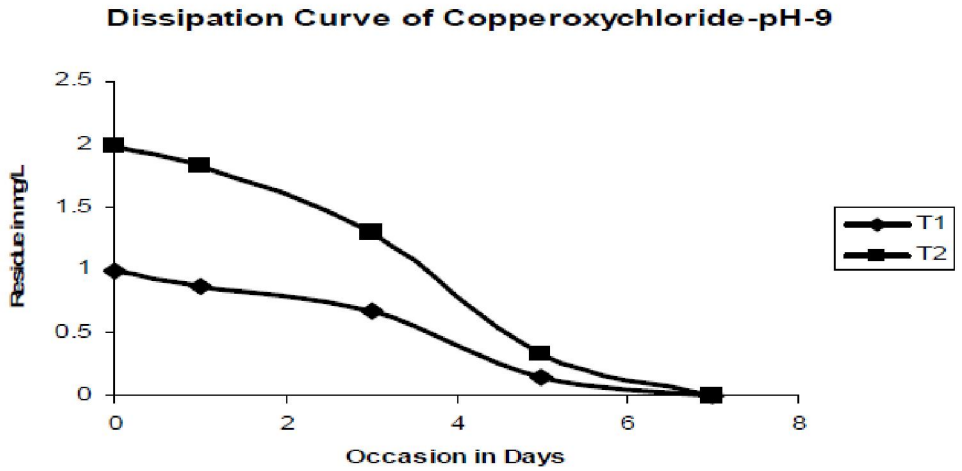

Figure 7 : Dissipation curve of copper oxychloride in basic water

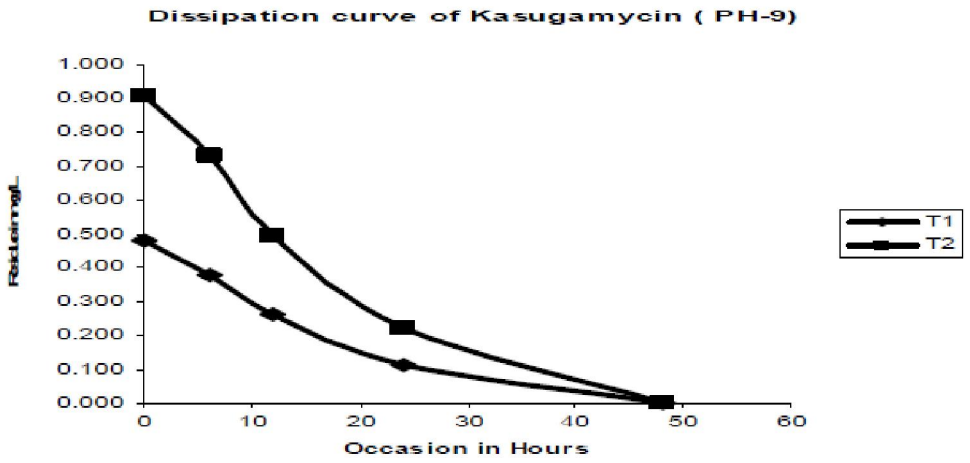

Figure 8 : Dissipation curve of kasugamycin in basic water

The DT50 (Half Life) of copper oxychloride and Kasugamycin calculated by regression analysis from the dissipation data.

CONCLUSION

Satisfactory validation parameters such as lin-

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earity, recovery, precision and LOQ and DT 50 values were established by following South African National Civic Organization (SANCO) and Environmental Protection Agency (EPA) guidelines[13]. Therefore, the proposed analytical procedure and dissipation data could be useful for regular monitoring, residue labs and research scholars to determine the copper oxychloride and Kasugamycin residues in different commodities (crop, water and soil samples).

ACKNOWLEDGEMENTS

The authors are thankful to the Dr. B. Gowtham Prasad, SVV University, for providing necessary facility to conduct the Laboratory experiment.

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