International Journal of Applied Environmental Sciences
ISSN 0973-6077 Volume 15, Number 1 (2020), pp. 137-150

http://www.ripublication.com/ijaes.htm

Quantification of Ziram Using Malachite Green Dye in Agricultural Samples

Kaushilya Mannewar[1] & Dr. Kishore K. Tiwari[2]

Department of Chemistry, Govt. Nagarjuna P.G. College of Science, Raipur. 492010, Chhattisgarh, India.

Abstract

Acidic hydrolysis of ziram fungicide to forms carbon disulphide gas and ethyl diamine with zinc ion, carbon disulphide gas further react with ethanolic sodium hydroxide to form xanthat, xanthate further react with potassium iodate to librate free iodine, malachite green dye reacts with free iodine to formed leucomalachite iodine complex whose colour is wine red. The reaction medium was maintained at pH 2.5-3. Its maximum absorbance (λ_{max}) at 630nm, the reaction medium was carried out at 30-35ºC temperatures, the wine red colour solution were stabilized by N-chlorosuccinamide. The molar absorptivity was found to be 0.315 $lmol^{-1}cm^{-1}$Sandell's sensitivity was calculated to be 9.708×10^{-4} $\mu gcm^{-2.}$ The stastical treatment of the experimental results indicates the method is precise and accurate. The method has been successfully applied to the determination of ziram fungicides in various agricultural samples.

Keywords: Spectrophotometric; ziram fungicide, potassium Iodate, malachite green, sodium acetate trihydrate, acetic acid.

1. INTRODUCTION

At time being one of the fast developing worldwide problem is fungicide residues which are present in essential food items cause's health issues on human beings [1].Estimation of fungicides to human hazard can be determined by the limits present in the surroundings [2]. It has been reported that attack of pathogenic moulds in radish, carrot, bottle gourd so that cultivator used fungicides practically in frequent for protecting of their crops [3].It is appropriate to develop raise awareness to user for certainty that to known how much quantity of fungicides present in their edible product [4].The dithiocarbamate having thiocarbonyl group such as ziram which are frequently used fungicides against wide range of domestic and vegetable garden moulds [5].It was determined that dithiocarbamate fungicides i.e. ziram act as neurotoxin and attack to nervous system of various moulds [6].The varieties of procedure have been used for the determination of dithiocarbamate fungicide [7].These including HPLC-MS, TLC, GC-MS, AAS, NMR, FTIR, IR, Polarography, Spectrophotometry, several spectrophotometric methods have also been reported for the determination of ziram fungicide [8].The aim of proposed work is to develop simple, rapid and precise methods for analysing for the determination of globally used ziram fungicide at micro gram levels [9].The developed method is based on acidic hydrolysis of ziram fungicide to carbon disulphide gas, further react with ethanolic sodium hydroxide to form xanthate, xanthate further react with potassium iodate to librate free iodine and this librated iodine selective oxidize malachite green to leucomalachite iodine complex[10].The developed method has been successfully applied to the determination of ziram in agricultural samples [11]

2. EXPERIMENTAL SECTION

2.1 Apparatus:-A Systronics UV-Vis spectrophotometer 104 with 2cm matched silica cells was used for all spectral measurements. Systronics pH meter model No.335 was used for pH measurement.

2.2 Reagents:-All reagents were of analytical reagent grade and double distilled deionised water was used throughout the experiment.

2.3 Ziram solution:-2µg ziram was dissolved in 30ml of volumetric flask in acetone were prepared. The required working standard solutions were prepared by appropriate dilution of the stock.

2.4 Malachite Green dye:-0.05mg of malachite green dye dissolved in 100 ml distilled water.

2.5 Sodium hydroxide:-5% (5g/100ml) ethanolic sodium hydroxide solution was prepared as an absorbing solution for CS_2.

2.6 Potassium iodate (Merk):-0.1M solution was prepared by dissolving 0.713g of KIO_3 in 100ml water.

2.7 N-chlorosuccinimide solution (Aldrich):-250 mg of N-chlorosuccinimide was added to 250ml volumetric flask containing 2.5g of succinimide and volume was made up to the mark with water.

2.8 Acetate buffer solution:-13.6g (1M) sodium acetate trihydrate in 80ml of water, solution pH was adjusted 2.5-3 with acetic acid, and the mixture was diluted to 100ml with water.

2.9 Sodium salt of EDTA:-5% (5g/100ml) dissolved in distilled water, metaphosphoric acid 3% (3g/100ml) dissolved in distilled water.

3. PROCEDURE

A known amount of working standard solution of 2.5ml of 2µg per 30 ml of ziram prepared in acetone was introduced in the digestion flask of digestion-absorption apparatus [12]. Then 5M H_2SO_4 was added drop wise to the above solution for hydrolysis during which CS_2 was released 5ml of ethanolic sodium hydroxide solution was taken as an absorbing solution [13]. Experiment was carried out for about 3-4 min, the pale yellow colour xanthate formed was diluted with water as required; 2 ml of ziram solution taken in a 20ml calibrated test tube; to it 2ml acetate buffer solution, 2ml potassium iodate, 1ml of N-chlorosuccinimide were added and gently shaken for few seconds; 2ml of malachite green solution was added to the solution; pale yellow colour solution turn into wine red colour solution [14].The reaction mixture was neutralize slightly alkaline with 3% of metaphosphoric acid with sodium salt of EDTA solution was added then mixture was kept for 5 min for completion of the reaction; the absorbance of formed complex was measured at 630nm against the reagent blank; the concentration of ziram content was established from the calibration graph [15].

4. RESULTS AND DISCUSSION

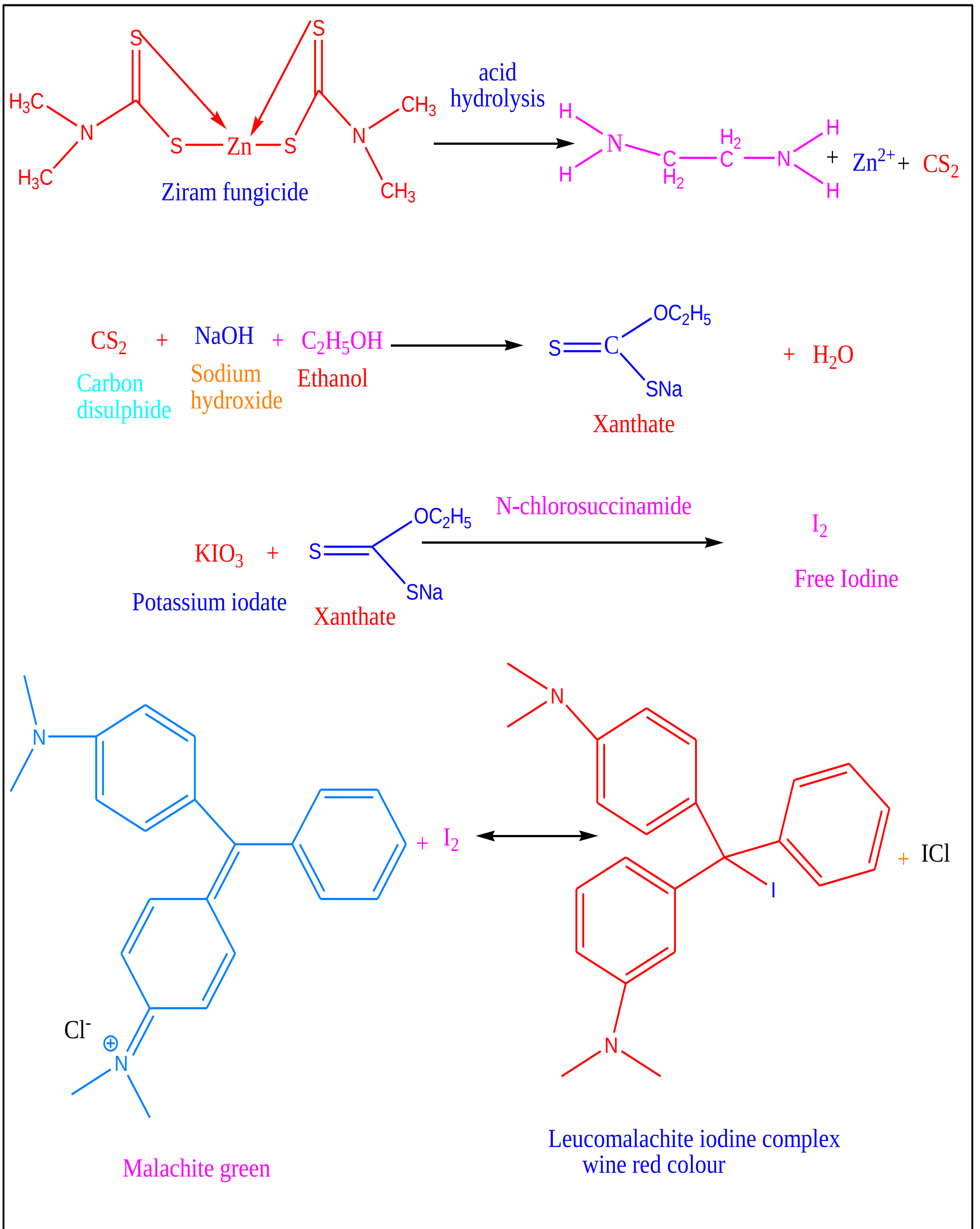
4.1 Colour reaction of Ziram:-

1) When acid hydrolysis of ziram fungicide occurs then ethylamine; CS_2 and Zn^{2+} ion are formed [16].

2) CS_2, NaOH and C_2H_5OH reacts together to form xanthate and H_2O.

3) Xanthate reacts with oxidizing agent KIO_3 in presence of N-chlorosuccinimide to librate free I_2. Potassium iodate has +5 oxidation states it changes into zero oxidation state in free iodine. Here iodine is selectively oxidized [17].

4) Malachite green react with I_2 to form leucomalachite iodine complex whose colour is wine red shown in (Scheme-1). Free iodine has zero oxidation states it changes into +1 oxidation state in ICl. Here malachite dye is reduced.



Scheme-1: Schematic proposed representation of Leucomalachite iodine complex (wine red colour)

4.2 Absorption Spectra of Ziram:- Absorption spectra plotted between wavelength on x-axis and absorbance on Y-axis. (a) Maximum absorbance is obtained at 630nm. (b) It shows absorption spectra of reagent blank.

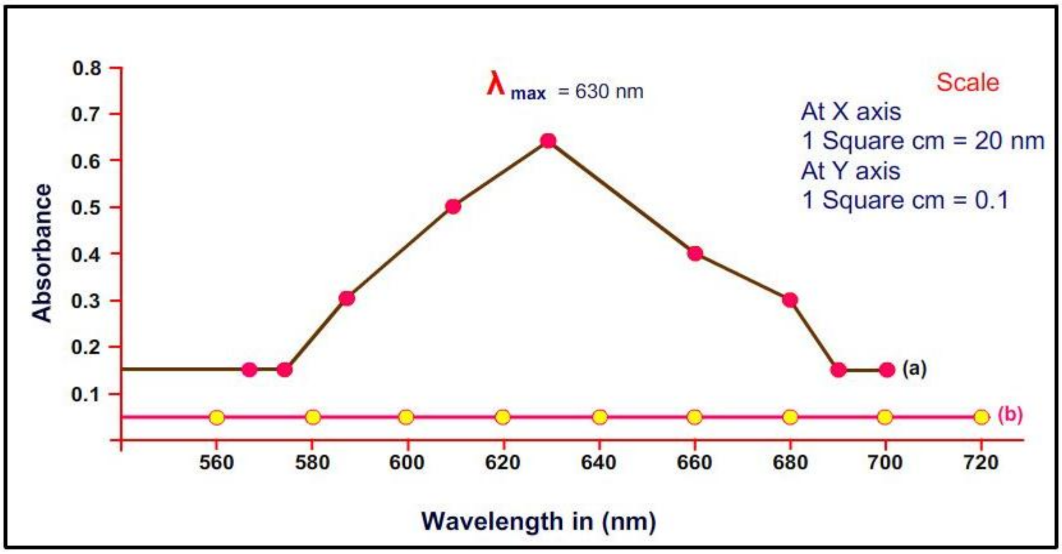


Fig.1-Absorption spectra of fungicide (a) Absorption spectra of ziram fungicide with malachite green dye; (b) Absorption spectra of reagent blank

4.3 Calibration Curve of Ziram:-The graph is plotted between concentration of ziram fungicide in (μg) on x-axis and absorbance on y-axis. A straight line is obtained. It follows Beer-Lambert's law at range 0.09-0.73μg.

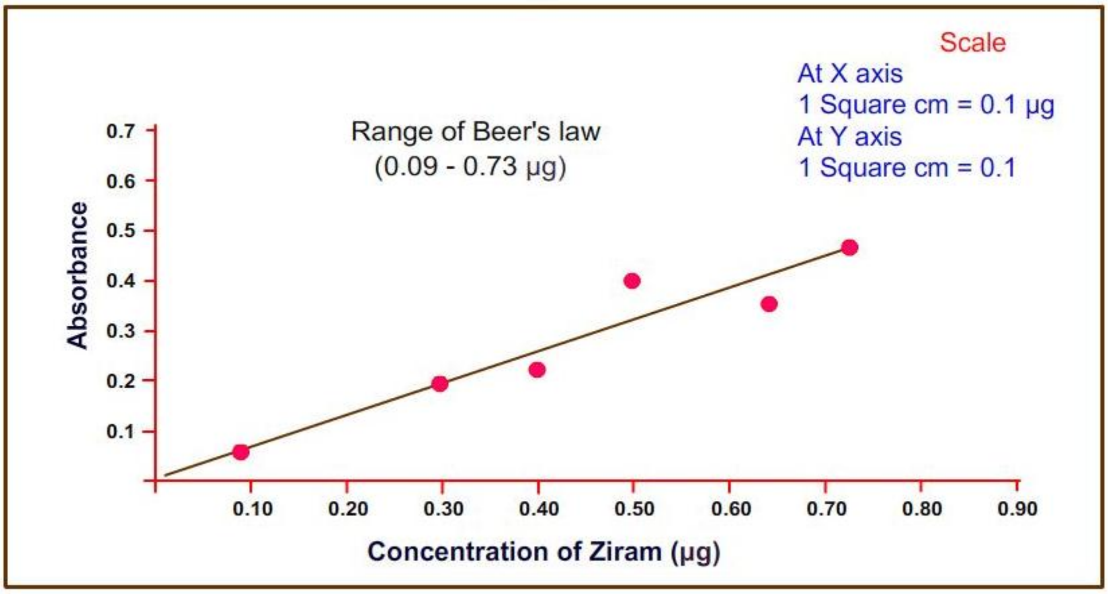


Fig.2-Calibration curve of ziram concentration level (1μg per100ml) with malachite green dye (0.05mg/100ml)

4.4 Effect of pH:-The graph is plotted between pH on x-axis and absorbance on y-axis. Colour development occurs at a pH range of 2.5-3.

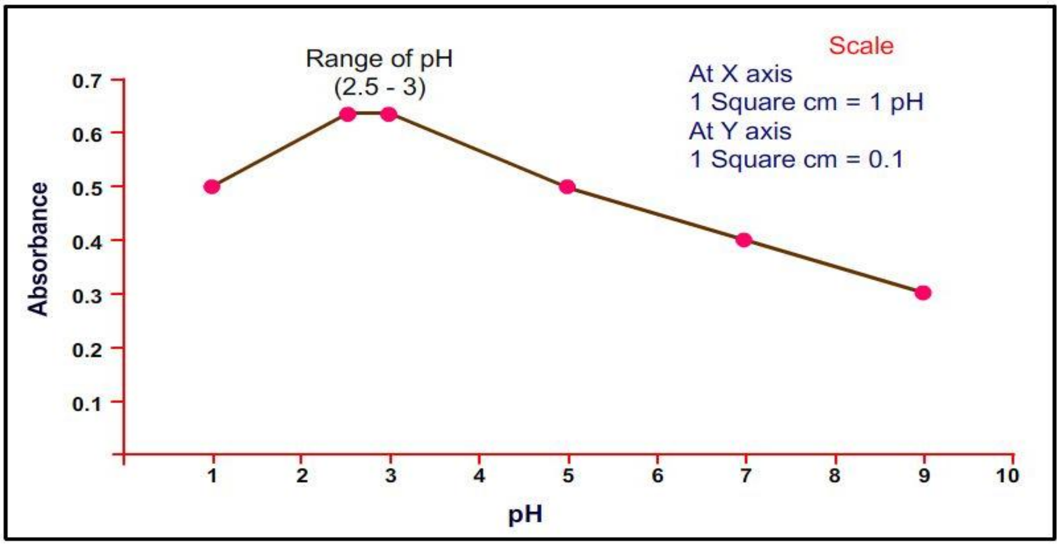


Fig.3-Effect of pH on absorbance of fungicide at concentration level of ziram (1µg/100ml) with malachite green dye (10mg/100ml)

4.5 Effect of Temperature:-The graph is plotted between temperature on x-axis and absorbance on y-axis. Temperature range of 30-35 ºC is essential for colour development.

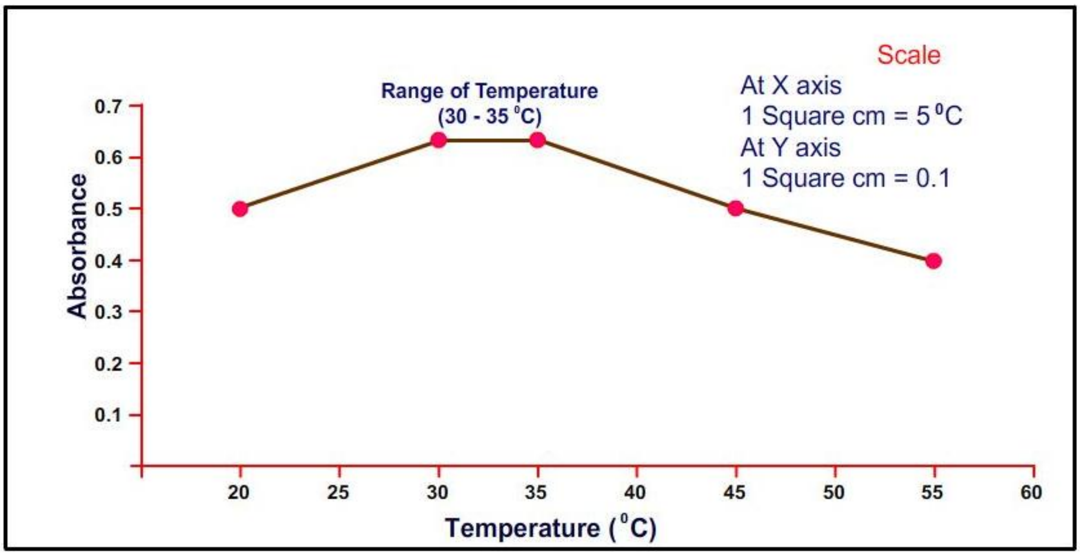


Fig.4-Effect of temperature on absorbance of concentration level of ziram fungicide in (1µg/100ml) with malachite green dye (10mg/100ml)

4.6 Effect of concentration Potassium iodate:-The graph is plotted between concentration of potassium on x-axis and absorbance on y-axis. 1.5ml of potassium iodate is sufficient for colour development.

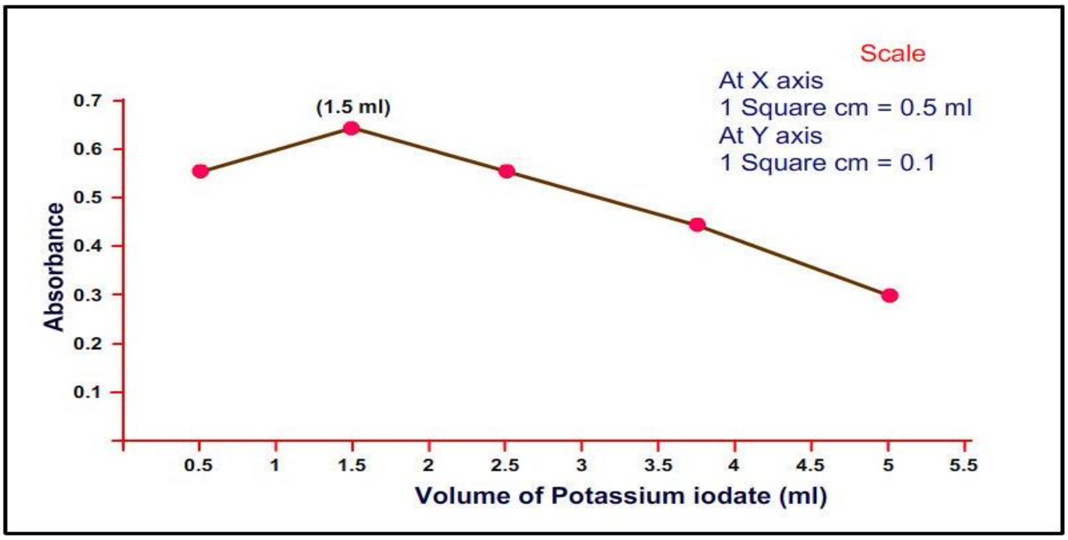


Fig.5: Effect of concentration of Potassium iodate1.5ml (0.713g/100ml) on absorbance of fungicide at concentration level of ziram (1µg/100ml)

4.7 Effect of concentration of malachite green dye:-The graph is plotted between concentration of malachite green on x-axis and absorbance on y-axis. 1.5ml of malachite green is important for colour development.

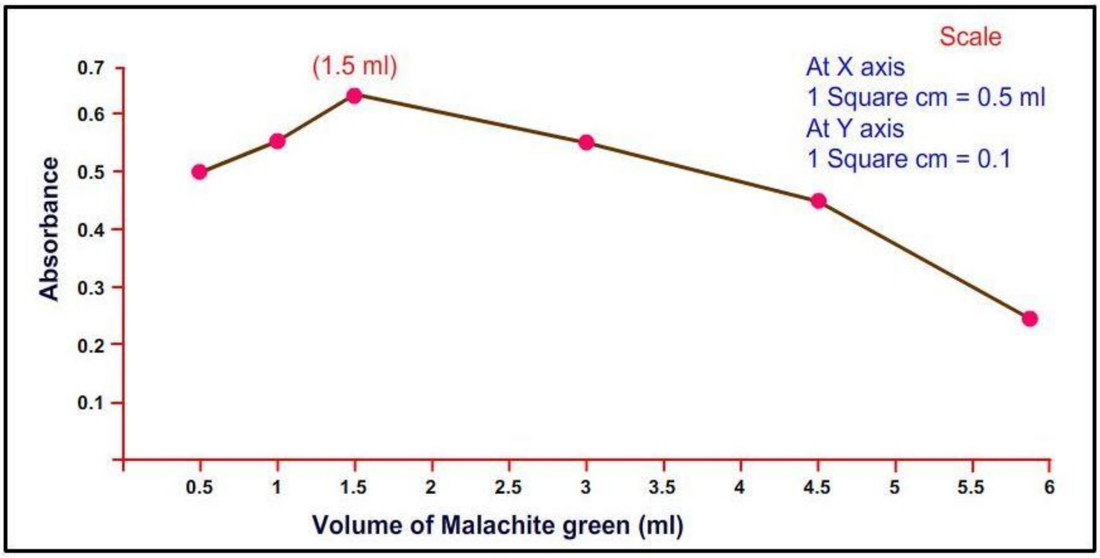


Fig.6: Effect of concentration of malachite green dye 1.75ml (10mg/100ml) on absorbance of fungicide at concentration level of ziram (1µg/100 ml)

4.8 Effect of concentration of Sodium Hydroxide:-The graph is plotted between concentration of sodium hydroxide on x-axis and absorbance on y-axis. 1ml of NaOH is sufficient for development of colour and maximum hydrolysis.

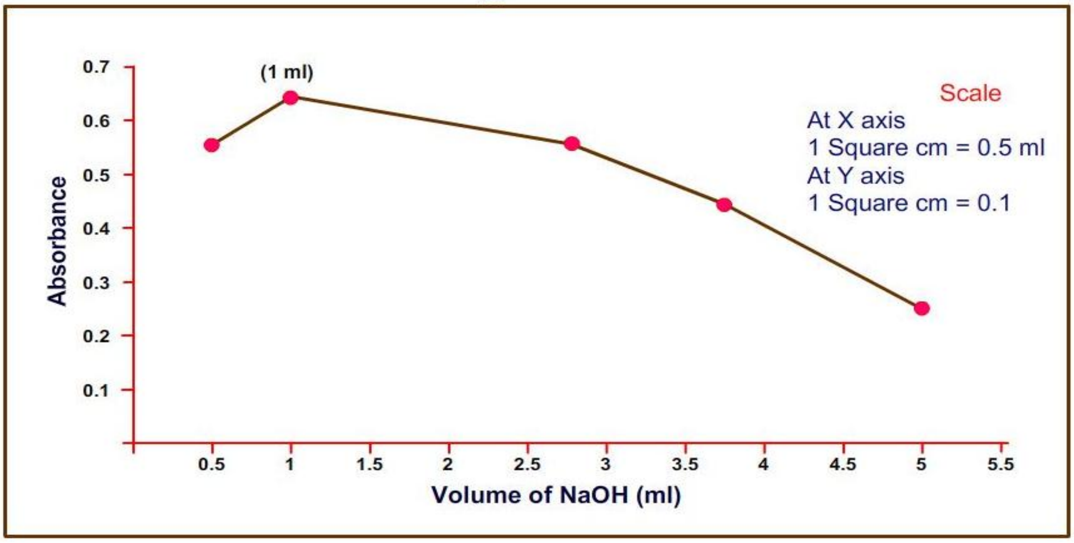


Fig.7: Effect of concentration of sodium hydroxide on absorbance of concentration level of ziram (1µg/100ml) with malachite green dye (10mg/100ml)

4.9 Effect of Concentration of ziram:-The graph is plotted between concentration of ziram on x-axis and absorbance on y-axis. 2ml of ziram is sufficient for colour development.

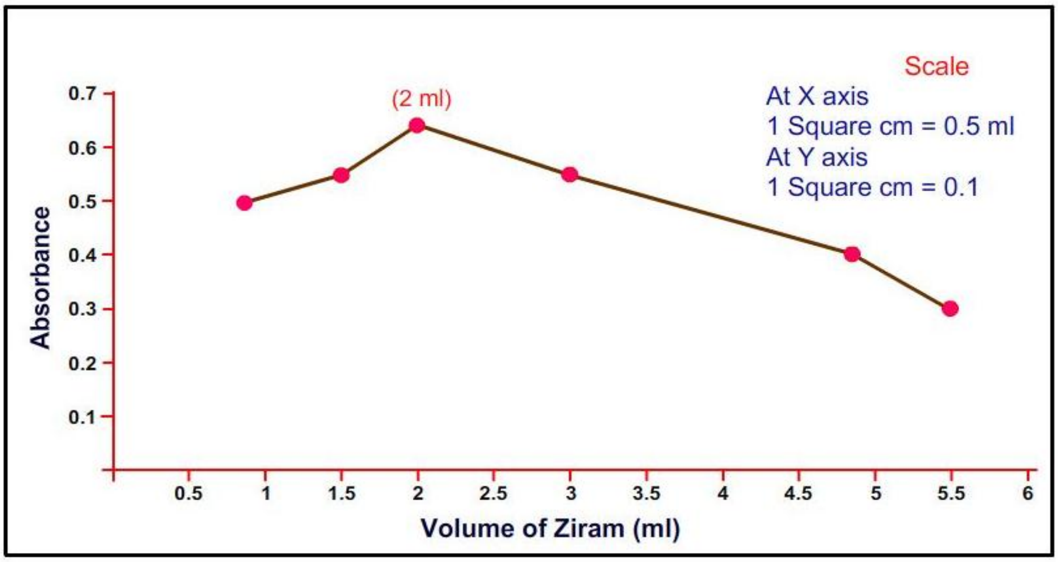


Fig.8: Effect of concentration of ziram 2ml (1µg/100ml) on absorbance of malachite green dye (10mg/100ml)

4.10 Spectral Characteristics (Table-1) all spectral characteristic shown in (table-1).

Parameters	**Ziram fungicide**
Maximum absorbance (nm)	630
Beer's law concentration range (µg)	0.09-0.73
Molar absorptivity (L mol-1cm-1)	0.315
Sandell's sensitivity (µgcm-2)	9.708×10^{-4}
Standard deviation	0.458, 0.3029
Correlation coefficient	0.970
Slope (m)	0.640
Intercept(C)	0
Limit of detection (µg/mL)	2.146
Limit of quantification (µg/mL)	4.732
Precision	0.02983±0.64
Accuracy	0.024831%
Absolute error	0.0247
Relative error	0.024831

4.11 Effect of foreign species

Effect of various interfering ions, pesticide, fungicides was studies to evaluate the validity of method in the analysis of ziram [18].Known amount of fungicides and metal ions were added to the sample solution and developed method used for the analysis of fungicide [19]. Presence of other commons ions does not interfere in the procedure but hydrogen sulphide and some ions like Cu^{2+}, Hg^{2+}, Fe^{3+} interfere with the determination of ziram; eliminated by its absorption in lead acetate, absorbance interfered in the determination of ziram(2µg/30ml) shown in (Table-2) [20].

Foreign Species	**Tolerance Limit(µg/ml)**	**Foreign Species**	**Tolerance Limit(µg/ml)**
Na^{+}, K^{+}, Ba^{2+}, Ca^{2+}	2000	Sulphate	380
Zn^{2+},Fe^{3+},Al^{3+},Cu^{2+}, Hg^{2+}	400	Phosphate	20
Thiram, dibam	1100	Oxalate	10
DDT, BHC	950	Citrate	25
Cl^{-}, Br^{-}, F^{-}	500,100,500	Metabisulfite	30

4.12 Comparison with other methods

The comparison between reported method and developed method the reported method are less sensitive, it required hydrogen sulphide generation by methylene blue and benzyl mercaptan method requires addition extraction step; the present method is highly sensitive and can be applied to real sample in the presence of ziram shown in (Table-3) [21].

S. No.	Method	Max (λmax) (nm)	Beer's Law range	Sensitivity	Reference
1	Diphenylcarbazone	520	0.12.9	0.08 μg/ml	8
2	Methylene blue method	655	3-22	6.8×10^3	9
3	Benzyl mercaptan	430	14-150	-	5
4.	Malachite green	565	0.68-0.81	9.74×10^5 $lmol^{-1}cm^{-1}$	Present method

4.13: Determination of ziram fungicides in Fruits, Vegetables, Runoff water of Farming

To check the validity of the method and determination of the ziram fungicide in 50ml agricultural water sample were taken from the fields where ziram has been sprayed as fungicide such as Pahanda and Torla[22]. These samples were found to be free from ziram; known amount of ziram is added in agricultural water sample taken in 100ml beaker 1.5ml of concentrated sulphuric acid mixed and pH maintained at 2.5-3 by the use of acetate buffer [23].These samples were analyzed through recommended procedure [24]. Various samples of vegetables, potato tubers, lettuce, green leafy vegetables, and wheat were collected from agricultural field where ziram, has been sprayed. 300g of cabbage and other vegetables, fruits taken and blended in a mixture; known amount of ziram are added and kept some days; the sample was digested with 100ml of sulphuric acid [25]. The mixture was filtered and analysed by recommended procedure shown in (Table-4) [26].

Samples	Fungicide Added (μg) A	Fungicide Found (μg) B	Recovery ,% (B/A) × 100
Ziram Rice	4	2.62	(2.62/4) × 100 = 65.5
	7	5.20	(5.20/7) × 100 = 74.28
Wheat	4	3.1	(3.1/4) × 100 = 77.5
	7	4.3	(4.3/7) × 100 = 61.42
Watermelon	4	2.70	(2.70/4) × 100 = 67.5
	7	5.09	(5.09/7) × 100 = 72.71

Samples	Fungicide Added (µg) A	Fungicide Found (µg) B	Recovery ,% (B/A) × 100
Guava	4	3.04	(3.04/4) × 100 = 76
	7	5.08	(5.08/7) × 100 =72.5
Banana	4	2.09	(2.09/4) × 100 = 52.25
	7	4.02	(4.02/7) × 100 = 57.42
Cucumber	4	3.05	(3.05/4) × 100 = 76.25
	7	5.06	(5.06/7) × 100 = 72.28
Bottle gourd	4	2.45	(2.45/4) × 100 = 61.25
	7	5.30	(5.30/7) × 100 = 75.71
Onion	4	2.62	(2.62/4) × 100 = 65.5
	7	5.24	(5.24/7) × 100 = 74.85
Farming Runoff water	4	2.70	(2.70/4) × 100 = 67.5
	7	5.07	(5.07/7) × 100 = 72.42
Potable water	4	2.85	(2.85/4) × 100 = 71.25
	7	5.01	(5.01/7) × 100 = 71.57

5. CONCLUSION

The proposed developed method is based on use of new reagent malachite green dye for the spectrophotometric determination of ziram fungicide. Proposed method offers simple sensitive, selective and affordable. This developed method is much better than other sophisticated equip mental methods. Impact on community to spread awareness, amongst community, that have this fungicide of harmful to human health and environment. To give suggestion to the local farmers for tempted used for this fungicide. In present scenario development of method for analysis of any harmful chemical is important technique, through many sophisticated instrumental methods are available but this classical method have their own importance forever. Significance of method was many analytical parameters have been studied, results as approved by statiscal analysis accuracy and precision are determined. Results of application reflect the reliability to the method. In the development method no any harmful, chemicals were used; the proposed method is reliable, precise, affordable and easily available in small laboratory then other sophisticated instrumental reported method.

ACKNOWLEDGEMENT

I express my gratitude to Principal Dr. D. N. Verma Govt. Nagarjuna P.G. College of

Science. Dr. Kishore K. Tiwari my guide Head of Chemistry Shahid Rajiv Pandey Govt. College Bhatagoan Raipur, Dr. A. K. Mishra Head, Department of Chemistry Raipur. I am also thankful to University Grant Commission, New Delhi for providing laboratory facilities and financial assistance to carry out this experiment.

REFERENCES

[1] Michaela S. Stokholma, Ednar G. Wulffb, Elisabeth P. Zidac, Ibié G. Thioc, James B. Néyac, Romain W. Soallac, Sylwia E. Głazowskaa, Marianne Andresena, Henrik B. Topbjergd, Birte Boeltd, Ole S. Lunda, DNA barcoding and isolation of vertically transmitted ascomycetes in sorghum from Burkina Faso: Epicoccum sorghinum is dominant in seedlings and appears as a common root pathogen 191 (2016) 38–50

[2] Sukumar Chatterjee, Ajay Pillai, V. K. Gupta School of Studies in Chemistry, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh 492010, India Spectrophotometric determination of mercury in environmental sample and fungicides based on its complex with *o*-carboxy phenyl diazoamino *p*-azobenzene *Talanta* 57 (2002) 461–465.

[3] 3. Huiqiong Yan, Xiuqiong Chen, Yuhong Feng, Fei Xiang, Jiacheng L Zaifeng Shi, Xianghui Wang, Qiang Lin, Huiqiong Yan, Xiuqiong Chen, Yuhong Feng, Fei Xiang, Jiacheng Li, Zaifeng Shi, Xianghui Wang, Qiang Lin, Modification of montmorillonite by ball-milling method for immobilization and delivery of acetamiprid based on alginate/exfoliated montmorillonite nanocomposite, (2016) 73, 1185–1206.

[4] S. Kanchi, P. Singh, K. BisettyDithiocarbamates as hazardous remediation agent: A critical review on progress in environmental chemistry for inorganic species studies of 20th century Department of Chemistry, Durban University of Technology, *South Africa Arabian Journal of Chemistry* (2014) 7, 11–25.

[5] Baljit Singh, D. K. Sharma & Abhishek Dhiman Environment friendly agar and alginate-based thiram delivery system *Toxicological & Environmental Chemistry*, (2013) Vol. 95, No. 4, 567–578.

[6] European Commission (EC), SEC (2007) 1411, Monitoring of pesticide residues in products of plant origin in the European Union, Norway, Iceland and Lichtenstein (2005) EC, Brussels, Belgium, (2007). Mosby, USA, (1998).

[7] S. Rastegarzadehn, Sh. Abdali Colorimetric determination of thiram based on formation of gold nanoparticles using ascorbic acid *Talanta* 104 (2013) 22–26

[8] European Commission (EC), SEC (2007) 1411, Monitoring of pesticide residues in products of plant origin in the European Union, Norway, Iceland and Lichtenstein (2005) EC, Brussels, Belgium, (2007).

[9] Edwards, I. R., D. G. Ferry and W. A. Temple. (1991), Fungicides and Related Compounds, In Handbook of Pesticide Toxicology, Vol.3, Classes of

Pesticides. Wayland J. Hayes and Edward R. Laws (eds.) Academic Press, NY.

[10] Jegannathan Kenthorai Raman, Edgard Gnansounou Furfural production from empty fruit bunch – A bio refinery approach Industrial Crops and Products 69 (2015) 371–377

[11] M. A. Hernández-Olmos, L. Agüí, P. Yáñez Sedeño, J. M. Pingarrón, *Electrochim. Acta* 46, (2000), 289–296.

[12] R. Kesari, V. K. Gupta *Talanta* 45 (1998) 1097-1103.

[13] D. Chem, Q. C. Cui, H. Yang, Q. P. Dou, Cancer Res. 66(2006) 10425.

[14] Vaneet K. Sharma, jatinder S. Aulakh, Sonam Bansal, Ashok K. Malik and Rakesh K. Mahajan. *Intern. J. Environ. Anal. Chem.*Vol-84, no14-15,1105-1110, (2004).

[15] Olalla López-Fernández, M. Fátima Barrosoa, Diana M. Fernandes, Raquel Rial-Oterob, Jesús Simal-Gándara, Simone Morais, Henri P. A. Nouws, Cristina Freire, Cristina Delerue-MatosVoltammetric analysis of mancozeb and its degradation product ethylenethiourea *Journal of Electroanalytical Chemistry* 758 (2015) 54–58.

[16] G.Vettorazzi, W. F. Almeida, G.J. Burin, R. B. Jaeger, F. R. Puga, A. F. Rahde, F. G. Reyes, S. Schvartsman, Teratog. Carcinog. Mutagen, 15 (1995) 313.

[17] Abakerli,R.B.;Sparrapam,R.;Sawaya,A.C.H.F.;Luiz,A.J.B.;Galvao,T.D.L.;Martins,D.S.Y.;Yamanishi,O.K.;Toledo, H. H. B.; *Food Chem.* (2015),188,71

[18] Cereser, C., Boger, S., Parvaz, P., and Revol, A. (2001). Thiram-induced cytotoxicity is accompanied by a rapid and drastic oxidation of reduced glutathione with consecutive lipid per oxidation and cell death. *Toxicology*, (2001), 163: 153-162.

[19] E.H.F. Donald, Chemistry of the pesticides, 3rd Ed, pp 294-297. Van Nostrand, New York, (1955).

[20] Petrascu (S. Petrascu, et al. (1986), A. L. J. Rao et al (1989) and Rosentha et al (1953).

[21] J. Rosenthal, R. L. Carsor and F. L. Stanley, *J. Assoc. Agric. Chem.* (1953), 36, 1170.

[22] H. Martin, Pesticide Manual, Basic Information on the Chemicals Used as Active Components of Pesticides, firsted, British Corporation Protection Council. (2011)

[23] Health & Consumer Protection Directorate-General 2006Quality control procedures for pesticide residue analysis, document, *European Commission, Brussels* (2006)

[24] Malik, A. K.; Rao, A. L. J. Spectrophotometric determination of ziram, ferbam and zineb using diphenylcarbazone. *Talanta* (1991**)**, *8,* 941-944.

[25] D. G. Clarke, H. Baum, E.L. Stanley, W.E. Hester, *Anal.Chem.*23 (1951), 1842.

[26] European commission, Directorate-General for Health and Food Safety; Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides residues Analysis in Food (2015).