Section A-Research paper
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Determination of Thiram Using RGB Image Analysis and its Application in Soil and Vegetables

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Abstract: The proposed method describes the use of paptode for the determination of the thiram. The method describes chemo sensors developed for the determination of the thiram. The method is based on the thiram is hydrolyzed by HCI and change it in dimethylamine, which are coupled with NEDA and resulting pink magenta color azo dye formed on the paper platform. The color system formed pattern on an inert support. Scanner detects the product on TLC strip and obtained analyzed with program written in visual basic. The proposed sensor in linear in concentration range 100-1000 µg/ml. All analytical parameters and optimum reaction condition was evaluated. The sensors are use to detect, thiram in red sample i.e., soil and vegetable.

Keyword: paper paptode,scanner,tlc strips

INTRODUCTION

Thiram is a dimethyl dithiocarbamate, a wide-spectrum, non-systemic, fungicide [1]. It is a type of sulphur fungicide. Which is complete soluble in chloroform, acetone, and ether. It is available as dust, flowable, wettable powder, water dispersible granules, water suspension formulations and in mixtures with other fungicides [2]. It is nearly immobile in clay soils or in soils with high organic matter. Its melting point 155-156°c, boiling point 129°c, flash point 138°c, vapour pressure less than $7.5* 10^{-6}$ mm Hg at 25ºc, it is slightly soluble in water at 30 mg/ l at25ºc, specific density 1.29(20°c), and is stable in acidic media Some deterioration may occur on prolonged exposure to heat, air or moisture. [3-7]

Thiram is used to prevent seed and crops from fungal diseases like small seeded vegetables, large seeded vegetables, cereal grains and other seeds, coniferous seeds, cotton seed, ornamental seeds, and soya beans. It is also used as an animal repellent to protect fruit trees and ornamentals from damage by rabbits, rodents and deer. It is effective against Stem gall of coriander, damping off, smut of millet, neck rot of onion, etc. Thiram has been used in the treatment of human scabies, as a sun screen and as a bactericide applied directly to the skin or incorporated into soap [1,8-10]

It is used as a vulcanizing agent in the rubber industry. It can be also present in the environment as an oxidation product of two other widely employed fungicides, ferbam and ziram and it can persist in soil for several weeks [1]. Thiram is a non-systemic fungicide used to prevent crop damage in the field and to protect harvested crops (apples, peaches, and strawberries) from deterioration in storage or transport.

Various analytical methods have been reported by the scientists for qualitative and quantitative determination of thiram. These method are based on spectrophotometric (11,12), emission spectroscopy(13), liguid chromatography(14).), and flow injection chemiluminescence technique

(15,16)and High performance liquid chromatography (17), have also been reported. These methods are time consuming, expensive and specialized personomrel required in these techniques.

In the proposed method, thiram forms an azo dye by acidic hydrolysis followed by coupling reaction. The color spot has been developed on paper platform i.e., paptode. The color developed then digitized using image processing tool of MATLAB software. The method has several advantages over other existing method. The reactions parameters have been optimized such as reagent concentration, time, temperature, reproducibility+ and effective intensity for Red, Green and Blue band.

EXPERIMENTAL SECTION

APPARATUS AND SOFTWARE

In the proposed method Digital RGB analysis commercially available flatbed-scanner HP SCANJET G2410 is used for obtaining the images of color spots. Area of the spots, which were used to measure the color intensity, was a square with 60000 dpi (300_300 dpi). Resolution of the scanner was regulated at 300 dpi. For analyzing color values in RGB (red, green, blue) system, the software, which was written in visual basic media, was used. A mircolit-mircopipate was used for injecting samples paptode.

CHEMICALS AND REAGENTS

All A.R. grade chemicals and double distilled water was used throughout the experiment**.**

Thiram solution: 2500µg/ml stock solution of thiram was prepared by dissolving 0.25 g of thiram in 100ml solution of chloroform and HCl mixture(75ml chloroform solution and 25 ml of 2% of HCl)

NEDA *(N-(1-Naphthyl)ethylenediamine):* 3% solution of NEDA prepared by dissolving in distilled water

PROCEDURE

To construct the strips i.e., paptodes for thiram, strips of TLC were dipped into 3% concentration of the NEDA for few seconds and dried in a preheated oven at 40-50°c. Aliquots of 6 µl thiram solution injected on these TLC strips. Then the strips were dried again at 80-90°c in a preheated oven, A pink magenta color spot appears on the strips depending upon the concentration of thiram. The strips were scanned and images of spots were analyzed by image processing tool of MATLAB software system for finding their R, G and B values, then calculating respective intensities using standard formula. To obtain calibration curves, effective intensities of R, G and B values were plotted with respect to analyte concentrations.

CHEMICAL REACTION

In the proposed method thiram is hydrolyzed by hydrochloric acid and changes into dimethylamine and hydroxyl form of carbon di sulfide [34], which are coupled with NEDA and resulting pink magenta color azo dye formed on paper platform (Fig: 1)

PHOTOGRAPH

Photo shown colors appear by adding of thiram solution in paper platform. Different concentration of thiram injected on paptode, then developed different shades of pink magenta color on strips. Color will be darker then increasing the concentration of thiram.

RESULT AND DISCUSSION

Thiram formed pink magenta color azo dye product with NEDA. The proposed sensor is linear in concentration range of 100-1000µg/ml i.e.,6 µl injection volume containing 0.6 - 6 µg of thiram.

OPTIMIZATION OF THE REACTION CONDITIONS

Injection volume: The influence of volume of the analyte solution which must be injected onto the TLC strip was investigated. The optimum sample volume was obtained to be 6µl. With greater

injected volume spot not found perfectly and consequently the intensity of color was decreased (Fig: 2).

EFFECT OF NEDA

Different concentration of NEDA from 1%- 5% were prepared each solution immobilized on TLC strips then allowed to dry in a preheated oven at 40-50ºc. After drying 6 µl of standard thiram solution was injected on each TLC strips and corresponding effective intensity of the R, G and B values were plotted against concentration of NEDA solution (Fig 3).The maximum color intensity was observed at 3% solution of NEDA.

EFFECT OF TEMPERATURE

After immobilization of reagent onto the TLC Strips, the strips need to be dried. Various methods of drying may be applied such as at room temperature, in an oven and in hot air were applied and no change in signals was observed. However for increasing the speed of drying, a preheated oven is recommended in our study.

On preparation of paptode

The effect of temperature on paptode formation has been observed. The formation of paptode requied require 40-50° C temperature for drying, but below this temperature strips shown low intensity and above at it they become brittle and crused easily hence 40-50°C temperature has been recommended.

On development of spot

The effect of temperature on the color reaction has been studied (Fig: 4).The optimum temperature range for complete color reaction was found to be 80-90˚c. Below this, chemical reaction goes in another direction as it forms hydrogen disulfide [35] and above this temperature the color obtained was not homogenous.

CALIBRATION CURVES

The proposed system shows perfect linearity in the range from 100-1000 µg/ml i.e.6 µl injection volume containing 0.6 - 6 µg concentration of thiram for B band in RGB analysis as shown in Fig 7 while G band shows somewhat linearity but is not perfect linear where as R band does not show any linearity hence all the study has been carried out with B band in RGB analysis for the propose.

REPRODUCIBILITY,response time,stability,and detection limit of the system

Reproducibility of the proposed system has been investigated at seven separate paptodes with three replicate analyses over a period of 7 days under optimum condition for various concentrations of thiram. The values are illustrated in Table: 1.

The response time of the system was evaluated under optimum experimental condition 6 µl of 600 µg/ml (3.6 µg) of thiram. It was recorded by measuring the time required to achieve 95% values of color intensity of the spot. The response time of 2-3minutes was achieved.

To study the stability of color spots, 6 µl of 600 µg/ml (3.6 µg) of thiram was injected under optimum experimental condition on the paptodes. Scanning of the strips was done in the time period of 5, 10, 15, 20, 30, 60 120, 180, 240, 300,600 minutes. No change in color intensity was observed for a period up to 240 minutes that shows the color spot are stable for 4 hours after injecting thiram on paptode.

In order to study the stability of the sensors, after immobilization of NEDA on the TLC strips it was used periodically for 15 days, the signal did not show any significant change within 15 days of experiment. After 15 days strips change into slightly blackish forms and response time was found to be increased from 2-10 minutes this reveals that the strips are stable ~ 15 days.

Theoretical DL of this method was 0.10µg/ ml for R, 0.08 µg/ ml for G and 0.06 µg/ ml for B values. We also determine the detection limit by practical experiment. Practical DL is the lowest

concentration that would give a color on the TLC strip [36]. The practical DL for the proposed method was found to be about for 0.02

Chemical reaction

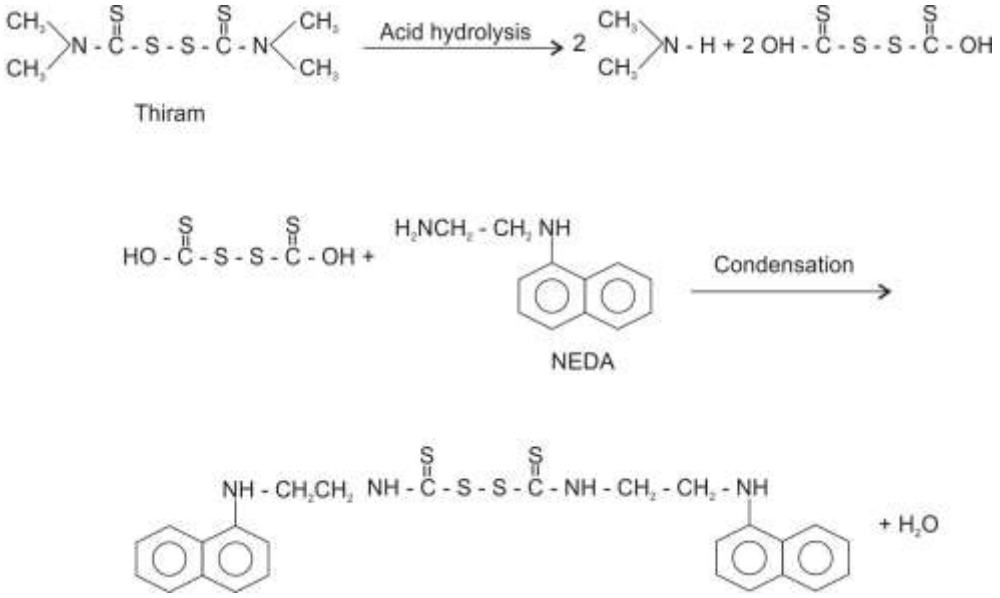
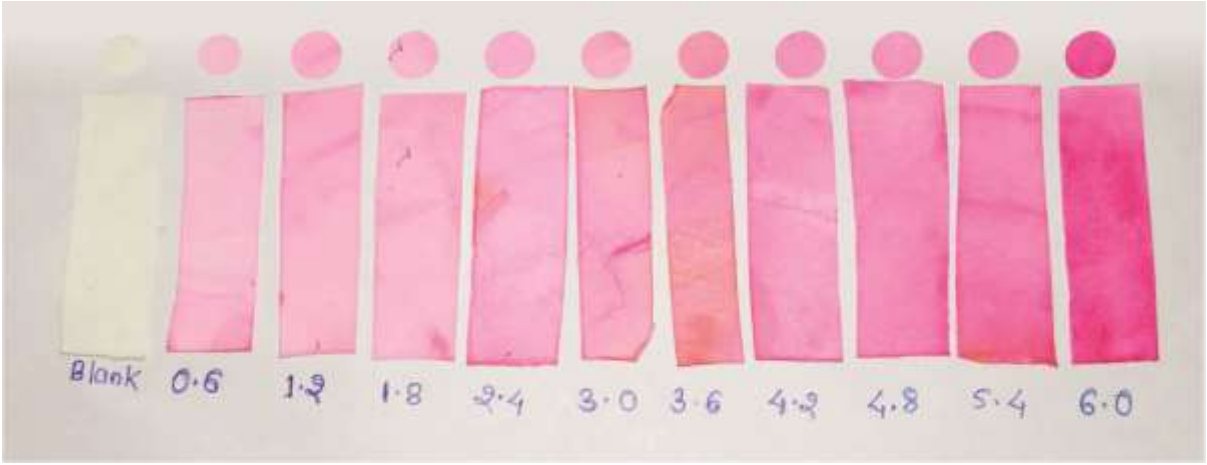


Fig: 1 Formation of pink magenta color azo dye



Photographs of paper paptode by adding different concentration of thiram with respect to blank

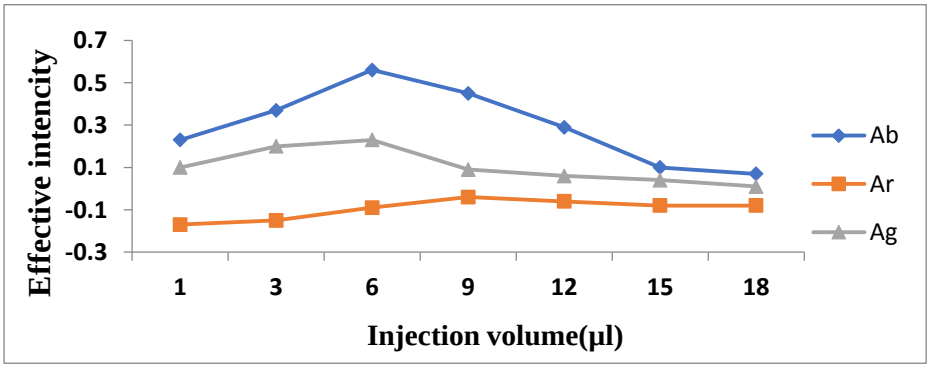


Fig: 2 injection volume of the analyte solution

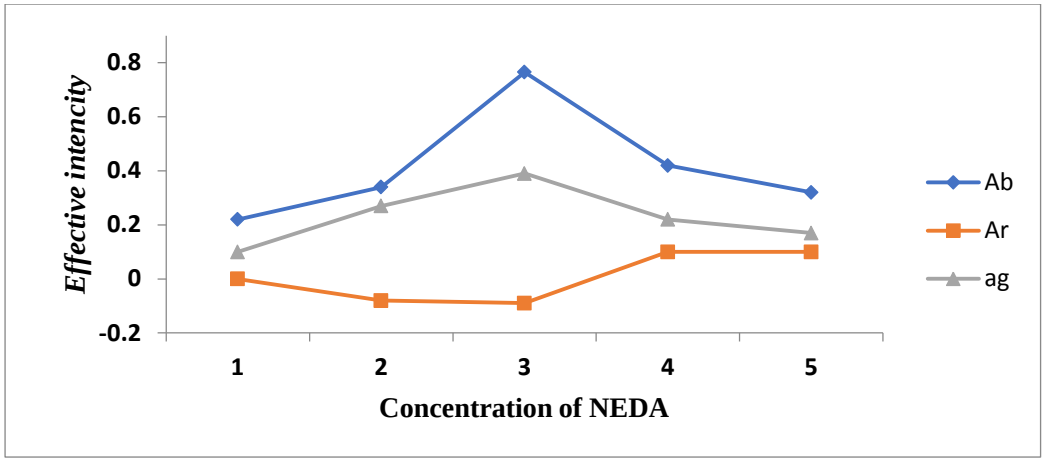


Fig: 4 Effect of concentration of NEDA

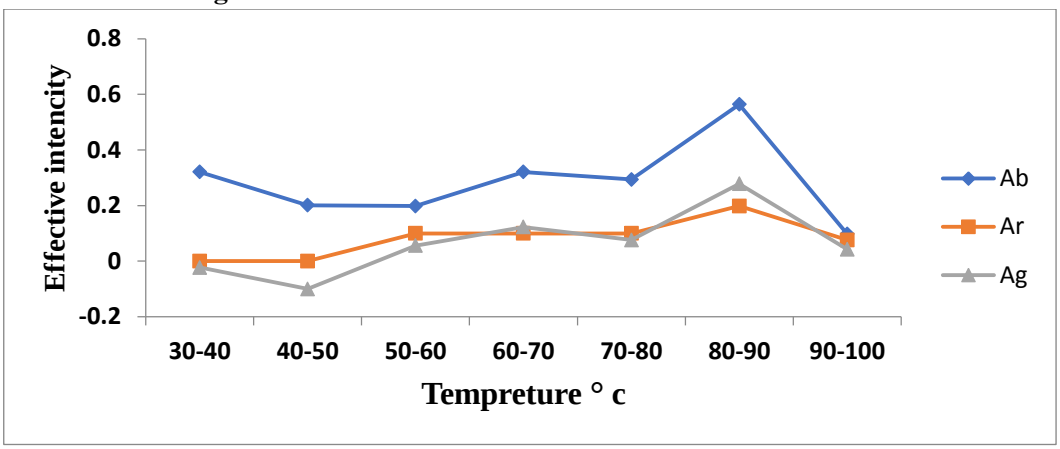


Fig: 5 Effect of temperature

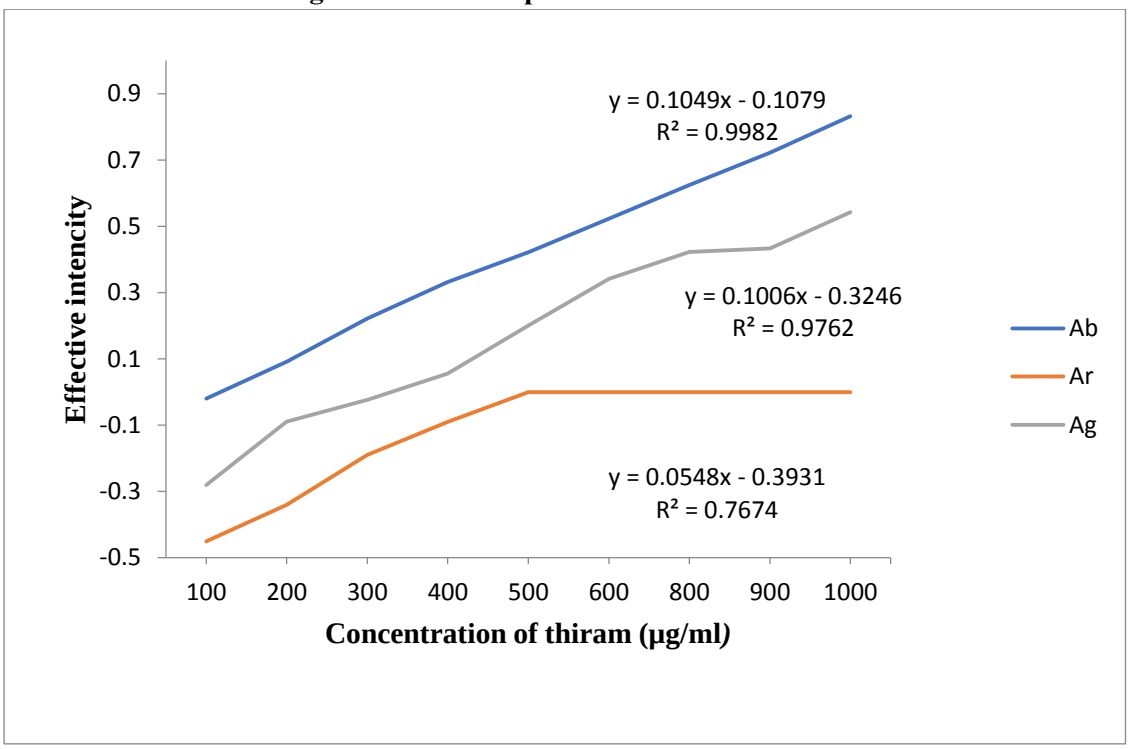


Fig: 7 Calibration graph of thiram(µg/ml) at optimum condition

Table 1 Reproducibility of the method

Concentration of thiram(µg/ml)	A_B			A_G		
	Ave[a]	SD[b]	RSD	Ave[a]	SD[b]	RSD
0.6	0.056	0.083	17..4%	0.037	0.06	11.6%
2.4	0.078	0.041	9.2 %	0.029	0.03	7.8%
3.6	0.145	0.06	6.3.%	0.038	0.03	3.1%
5.4	0.217	0.02	2.9%	0.123	0.01	2.1%

a Average of five measurement on different TLC

b Standard deviation

c Relative standard deviation

3.7 EFFECT OF INTERFERENTS

To check the validity of method the effects of various interferents have been studied under optimum experimental conditions for 6µl of standard solution containing 600µg/ml (3.6 µg) of thiram. The method was found to be free from most of the interferents including organic compound and metal ion in water. The tolerance limits for various interferents tested on paptode are shown in Table 2.

3.8 APPLICATION

The proposed method has been successfully applied for determination and sensing of thiram in grains, patato and soil sample.

In soil samples - The proposed method has been successfully applied for the determination of thiram in soil. A known amount of soil sample was collected from the agricultural field where thiram was sprayed as an insecticide. The sample was washed with 15 ml portion of 0.1 mol^{-1} EDTA twice and extracted with 2∗15 ml portion of chloroform [37]. The washing was collected. Aliquot of these washing were taken in a volumetric flask then analyzed by the reported method as well as by the proposed method. The results are shown in Table 3

In grains and potatoes –Patato tubers and grain such as rice and wheat collected from an agricultural field where thiram was sprayed as an insecticide. Samples were weighed, crushed into pulp, and washed with 2*15 ml portion of chloroform. The washing were collected and analysed by the proposed as well as reported method. To check the recoveries of the proposed method is 95-100%. Samples are also analysed with the reported method [37]. The results are reported in table 3

Table 2: Effect of foreign species on the determination of Thiram(600µg/ml)

Interferents	Masking agent	Tolerance limit (ppm)
Hydrazine		700
Methyl parathion		200
Diphenylamine , Nitrobenzene		130
Hydrazine		450
Paraquat, Endosulfan		600
Mancozeb		550
Ammonia,FAS		600
Phenol		400
Formaldehyde		150
Salicyldehyde		200
Ninhydrin benzeldehyde, pyridine		500
Cl^- , SO_4^-, CO_3^{2-} Na^+,K^+, Ca^{2+} , Mg^{2+}	10%EDTA solution(1ml)	1000
Cd^{2+}, Cu^{2+}	10%EDTA solution(1ml)	2500
Fe^{3+} /Al^{3+}	10%sodium potassium tartrate(1ml)	2500

a Causing an error of ±2% or less

Table 3: Analysis of Thiram in vegetable, grains and soil sample**

Samples	Thiram added(µg/ml)	Found by proposed method (µg/ml)	Recovery by proposed method (%)	Recovery by reported method (%) [37]
Rice	200	190.8	99.8	98.7
	300	300.00	100	99.0
	400	380.00	98.0	96.9

Wheat	200 300 400	200.00 270.20 390.99	100 97.00 99.99	98.6 97.0 97.2
Potato	200 300 400	180.98 290.00 400.00	98.50 99.00 100	96.0 98.9 95.7
Soil	200 300 400	200.00 260.99 390.00	100 95.2 98.00	99.9 94.9 95.2

*Amount of samples 1ml
**Mean of three replicate analyse

3.9 CONCLUSIONS

The method is simple, rapid, and user friendly and easy employable. It does not need any expensive apparatus. It's successfully applied for the determination of pesticides residue in grain and vegetable samples. The proposed method can be used for the determination of thiram in industrial health checkups and safety monitoring work. It is found to be better then other reported methods the comparison is given in Table 4.

Table 4:Comparison of the proposed method with some reported method

Technique	Linear range	Detection limit	Response time	Remark	Ref.
Spectrophotometry	24-100(µg/ml	0.3(µg/ml	_	pH dependent	11,12
Emmition spectroscopy	0.1-50(µg/ml)	0.016-0.086 (µg/ml		Carcinogenic reagent	13
Liguid chromatographhy	0.3-20mg/l	0.15 (µg/ml)	_	Based on redox peak -	14
flow-injection chemiluminescence system	0.1-1.0ppb 50-959ppb	0.04ppb	30min	Time consuming	15-17
HPLC	-	2.7ng/ml	_	Expensive instument	18
Proposed method	100-1000(µg/ml)	0.1(µg/ml)	25 min	Short response time, sensitive, user friendly	Proposed method

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