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Analytical Methods Section

Spectrophotometric determination of Maneb, Zineb and their decomposition products in some vegetables and its application to kinetic studies after greenhouse treatment

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A simple and accurate spectrophotometric method has been developed for the determination of Maneb, Zineb and their decomposition product, ethylenethiourea (ETU), using 2,6-dibromoquinone chlorimide (DBQ) and 2,6-dichloroquinone chlorimide (DCQ). The method depends upon releasing the ethylenebis-dithiocarbamate (EBDC) moiety from Maneb and Zineb using ethylenediamine tetraacetic acid disodium salt as a solvent. EBDC then reacts with either DBQ or DCQ yielding a red colour with a maximum absorption at 495 nm. ETU, on the other hand produces a yellow colour with a maximum absorption at 385 nm. The method was successfully applied to the determination of these compounds in cucumber and tomato fruits grown in greenhouses. The percentage extraction of Maneb and Zineb from cucumber was 94·5% and from tomato was 89·2%. The disappearance of Maneb and Zineb sprayed on cucumber and tomato grown in a greenhouse followed first-order kinetics. The rates of disappearance of Maneb and Zineb were correlated to the rate of growth of cucumber and tomato.

INTRODUCTION

Fungicide formulations containing ethylene bis-dithiocarbamate (EBDC) are widely used for protection of various crops against fungi (Bystricky & Notota, 1983). Maneb and Zineb are the main ingredients of these fungicides preparations. These compounds are currently used to control fungus on cucumber, green pepper and tomato grown in greenhouses in Egypt. The quantification and identification of the ethylene bis-dithiocarbamate fungicides have been a long-standing problem (Day & Hamilton, 1984). The official method for the analysis of EBDC formulations is based on evolution of CS_2 from hot acid-treated samples and iodine titration of the xanthine formed when the CS_2 is absorbed in alcoholic KOH (Horowitz, 1975; Hylin *et al.*, 1978).

Several techniques have been adopted for the determination of Maneb and Zineb, viz.: spectrophotometry (Hajslova *et al.*, 1988), differential thermal analysis (Day & Hamilton, 1984), GC (Bontoyan & Looker, 1973), GLC (Newsome, 1979) and HPLC (Smith *et al.*, 1988; Bardarov & Zaikov, 1989). Although EBDC is relatively non-toxic, its main metabolite and degradation product ethylenethiourea (ETU) (2-imidazolidinethione) (Rosenberg & Siltanen, 1979) was found to be carcinogenic (Graham *et al.*, 1973), goitrogenic (Graham & Hausen, 1972) and teratogenic (Horowitz, 1975). ETU may be present in EBDC formulations as a degradation product or on plants after application. ETU was determined using HPLC (Farrington & Hopkins, 1979; Vandamume *et al.*, 1981) GC (Bystricky & Notota, 1983), or after methanolic extraction. Methods of analysis of ETU in foodstuffs, biological fluids, and other substrates have been reviewed (Bottomley *et al.*, 1985). The aim of this work was to develop a simple and accurate spectrophotometric method for the determination of Maneb, Zineb and ETU residues on cucumber and tomato grown in greenhouses located in the Delta of River Nile, Egypt.

EXPERIMENTAL

Instruments

Absorption spectra were recorded using a Perkin Elmer 550s UV-visible Double-Beam Spectrophotometer. Vortex-SSC-P. SIBata SU-20, Dainichi Chemical Industrial Co., Ltd.

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Table 1. Performance data for the determination[a] of Maneb, Zineb and ETU with 2,6-dibromoquinone 4-chlorimide and 2,6-dichloro quinone 4-chlorimide

Compound	DBQ					DCQ				
	Concentration range ($\mu g\ ml^{-1}$)	% Recovery ± SD	Slope	Intercept	Correlation coefficient	Concentration range ($\mu g\ ml^{-1}$)	% Recovery ± SD	Slope	Intercept coefficient	Correlation
Maneb	2–14	99·93 ± 0·58	0·002 1	0·069 94	0·999 8	2–18	100·18 ± 0·70	−0·001 8	0·050 4	0·999 8
Zineb	2–25	100·32 ± 0·51	−0·003 3	0·040 9	0·999 8	2–35	100·49 ± 0·63	0·001 5	0027 3	0·999 8
ETU	10–100	99·93 ± 0·52	0·0107	0·010 70	0·999 7	10–120	9 977 ± 0·68	0·001 75	0·007 43	0·999 9

[a] The results are the average of eight determinations.

Materials and reagents

All chemicals used were of Analytical Reagent grade and solvents were spectroscopic grade. Maneb, Zineb and ETU were supplied by Wako Chemical Co., Tokyo, Japan. DBQ and DCQ (Aldrich) were 0·2% w/v in 2-propanol. EDTA (disodium salt (0·2 mol) solution was prepared. The pH of solution was adjusted to 9·8 using 0·2 M NaOH and the volume was made up to the mark with water.

Procedures

Preparation of standard curve of authentic samples

Preparation of standard solutions. Fifty mg of each of Maneb and Zineb were dissolved in 100·0 ml EDTA solution. Fifty mg of ETU was dissolved in 50·0 ml methanol.

Colour development. Increasing aliquots of solution containing the fungicide over the concentration range (Table 1) were transformed into a series of separating funnels. Then 0·2% DBQ or DCQ solution (1 ml) was added. The developed colour was extracted in *n*-butanol 3 × 5 ml), filtered onto anhydrous sodium sulphate into a series of 25 ml calibrated flasks and made up to the mark with the same solvent (*n*-butanol). A blank solution was prepared similarly using EDTA solution.

Preparation of standard curve of authentic samples in cucumber fruits

Crushed cucumber (5·0 g) was accurately weighed and transferred into 25 ml volumetric flasks. EDTA solution (10 ml) and methanol (5 ml) were added. Increasing aliquots of the standard solution containing the fungicide over the concentration range were added shown in Table 1. The samples were sonicated for 15 min and made up to the mark with water, filtered through Whatman filter paper No. 1 under suction. Ten ml of filtrate were transferred into 25 ml volumetric flasks. DCQ or DBQ solution (1·0 ml) was added and the procedure followed as for the standard curve.

Preparation of standard curve of authentic samples in tomatoes

Crushed tomato (5·0 g) was accurately weighed into 25 ml volumetric flasks. EDTA solution (3 ml) was added followed by methanol (5 ml). Increasing aliquots of the stock solution were transferred to each successive flask. The procedure as described before for the quantification in cucumber fruits was then followed.

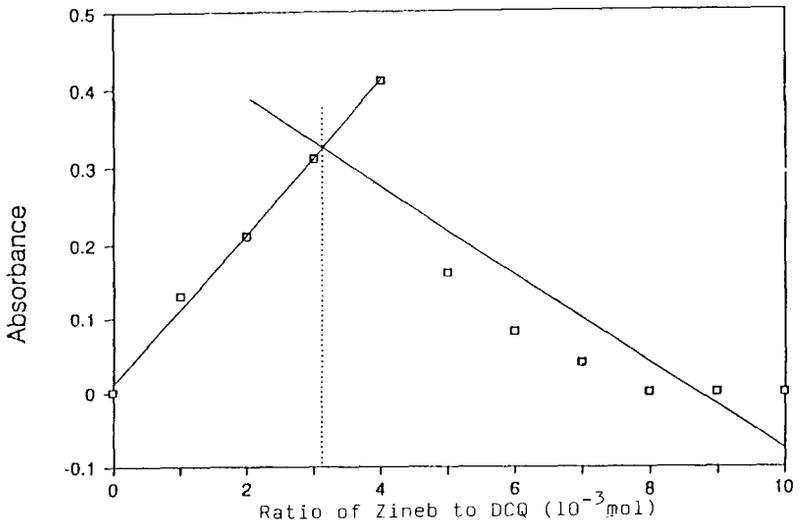


Fig. 1. Continuous variation plot of Zineb–DCQ reaction (10^{-3} mol).

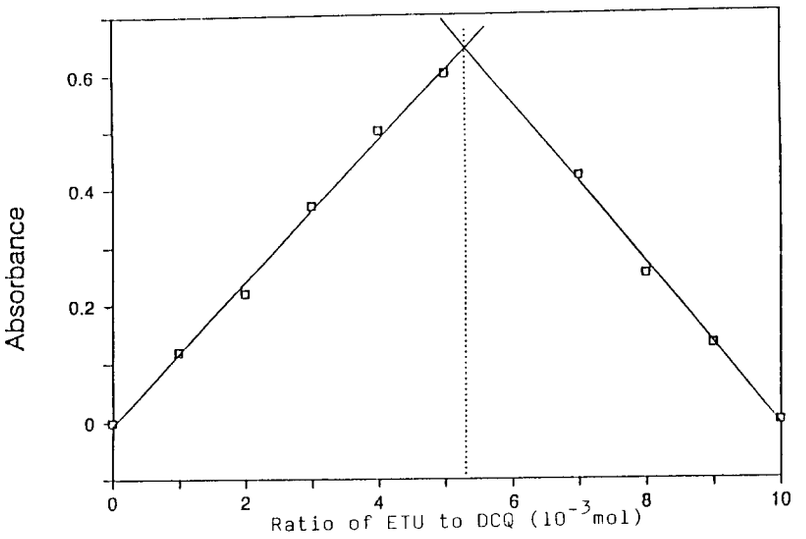


Fig. 2. Continuous variation plot of ETU–DCQ reaction (10^{-3} mol).

Field experiment

Location. The studies were conducted during winter and spring 1991 in separate 0·25 feddans (approx. 4200 m^3) of cucumber and tomatoes [Kesem F-1] grown in greenhouses located in Dakahillia Governorate, Egypt.

In one corner of the greenhouse, a plot containing 20 cucumber and tomato plants was isolated to serve as a control. The greenhouse was sprayed with Zineb formulation (Dithane Z-78) and another greenhouse was sprayed with Maneb formulation (Dithane M) at the recommended rate of 1 kg per feddan. For each treatment, the fungicide was applied in a suspension form in water using a Chapin No. 135 hand sprayer.

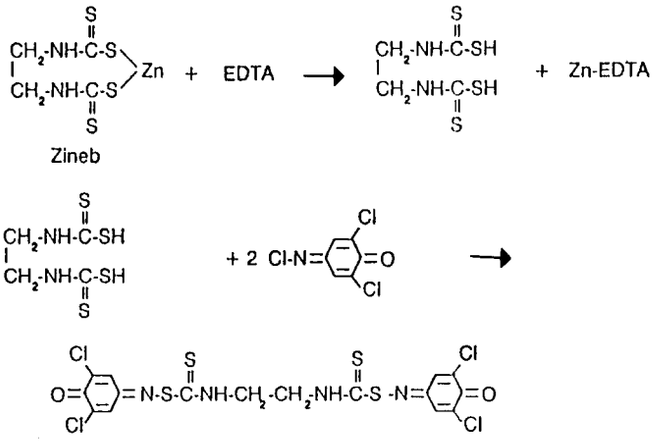
Sampling. Samples of cucumber with a length of more than 12 cm were collected 1 h before spraying, 1 h after spraying then at 12 h intervals. Samples were placed in plastic bags, and transferred in an ice box to the laboratory. Each batch of cucumbers was accurately weighed, and chopped and then frozen at −4°C until the day of analysis. Samples were thawed before use.

Quantification. Samples of cucumber equivalent to 5·0 g were transferred into a series of beakers and quantified as described in the preparation of standard curve in cucumber.

RESULTS AND DISCUSSION

The dihaloquinone chlorimides were found to react with Maneb, Zineb and their degradation products at pH 9·8 to give coloured reaction products with λ_{max} at 495 nm for Maneb and Zineb and 385 nm for ETU.

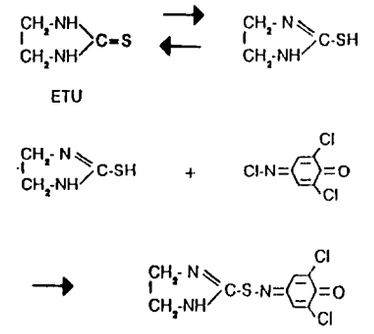
In order to study the reaction further, the molar ratio of DCQ to the studied compounds in the reaction mixture was determined by the continuous variation method (Rose, 1964). Figure 1 shows that ratio is 2:1 in the case of Maneb and Zineb and in the case of ETU is 1:1 (Fig. 2). On the other hand, according to Scudi (1941), DBQ or DCQ react via the chlorine atom in the chlorimide group. Accordingly, the reaction



Scheme 1. Proposal of the reaction between Zineb and 2,6-dichloroquinone chlorimide.

between the dihaloquinone chlorimide and the studied compounds proceeds as described in Schemes 1 and 2.

The reaction conditions were thoroughly studied. The problem of insolubility of these compounds in water was solved using EDTA (disodium salt) where the metal–EDTA complexes were formed and EBDC was released. As an alternative to EDTA, citric acid and tartaric acid were evaluated but the trial did not succeed. As for ETU, methanol was found to be a suitable solvent. Methanol, however, played another role



Scheme 2. Proposal of the reaction pathway between ETU and 2,6-dichloroquinone chlorimide.

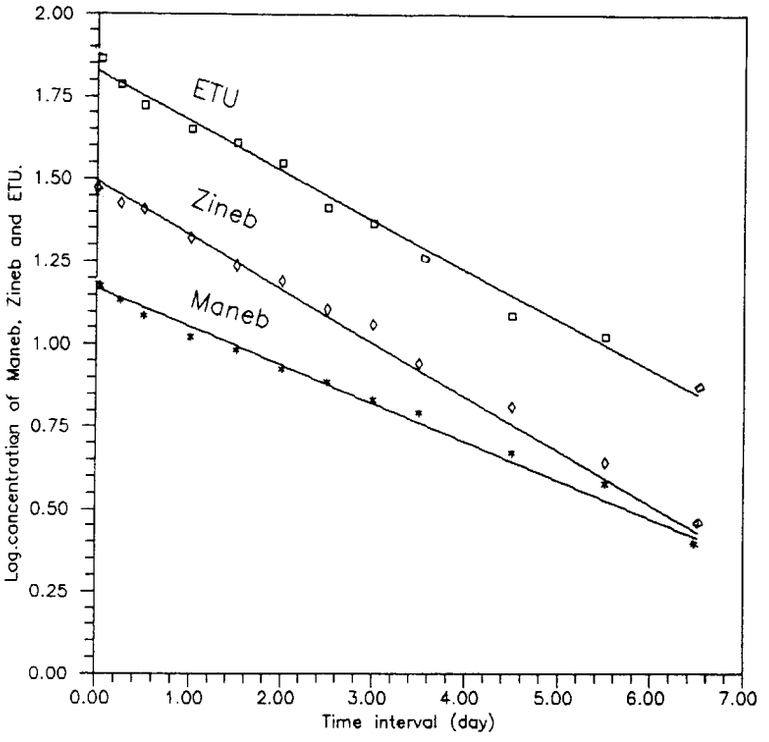


Fig. 3. First-order plot for Maneb, Zineb and ETU on cucumber fruits.

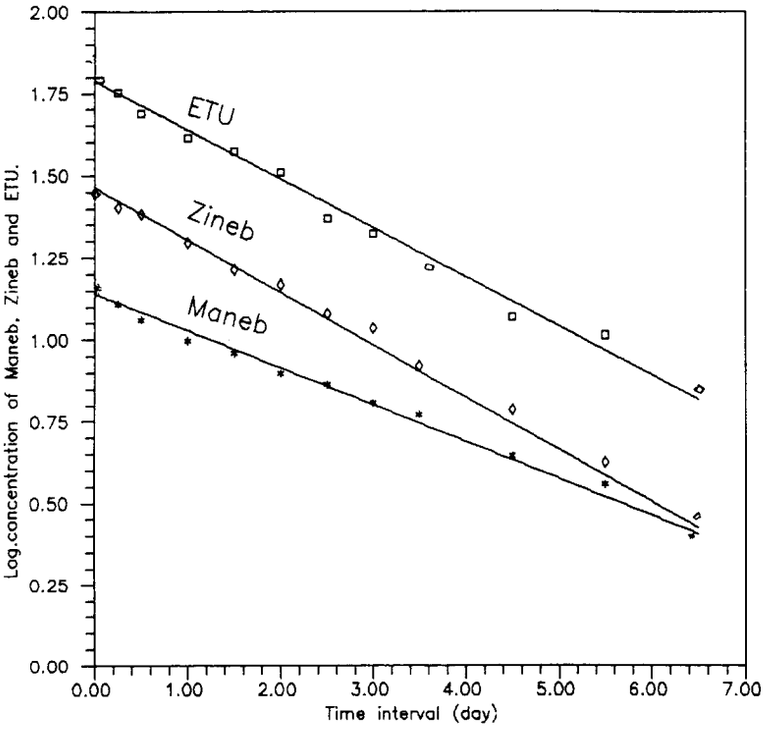


Fig. 4. First-order plot for Maneb, Zineb and ETU on tomato fruits.

Table 2. Residues of Maneb, Zineb and ETU on cucumber fruits after various intervals of treatment

Time (day)	Maneb (ppm)		Zineb (ppm)		ETU (ppm)		Change in cucumber weight[a]
	Proposed method	Official method[b]	Proposed method	Official method[b]	Proposed method	Official method[b]	
0·00	15·7	15·5	29·6	29·3	77·3	76·8	0·0
0·04	15·0	14·8	28·5	28·2	73·2	72·6	nd
0·25	13·6	13·8	26·7	26·4	61·0	60·5	nd
0·5	12·2	12·0	25·6	25·8	52·9	51·5	0·82
1·0	10·5	10·4	20·9	20·4	44·8	44·2	nd
1·5	9·6	9·8	17·3	17·6	40·8	40·1	0·61
2·0	8·4	8·2	15·6	15·2	35·4	34·6	nd
2·5	7·7	7·4	12·8	12·4	26·0	25·4	0·25
3·0	6·8	6·6	11·5	11·6	23·3	22·8	nd
3·5	6·2	5·8	8·8	8·6	16·5	15·6	0·13
4·5	4·7	4·5	6·5	6·3	12·3	11·7	0·09
5·5	3·8	3·6	4·4	4·1	10·6	9·9	0·03
6·5	2·6	2·3	2·2	2·1	8·4	7·5	0·03

[a] Ratio between the weight of cucumber fruit on application day to the weight on harvesting day.
nd, No determination was made.
[b] From Hylin *et al.* (1978).

in precipitating the indigenous compounds of the plant material. The developed colours were unstable in aqueous solution, decomposing within 15 min. Extracting the colour with 1-butanol renders it stable for several hours. Table 1 shows the performance data for the proposed method. The method was successfully applied for the determination of these compounds in cucumber and tomato fruits grown in greenhouses.

Maneb, Zineb and ETU were extracted from the fruits by sonication with EDTA and methanol. The percentage extraction of Maneb and Zineb from cucumber was 94·5% and from tomato was 89·2%. The percentage extraction of ETU from cucumber and tomato was 98·4.

The residues of Maneb, Zineb and ETU found in cucumber and tomato are listed in Tables 2 and 3. Analysis of the treated cucumber and tomato reveals the disappearance of any detectable EBDC after 6 days of spraying. The disappearance of Maneb and Zineb sprayed on cucumber and tomato grown in greenhouses were first-order in nature with regard to the concentration of Maneb and Zineb. Comparing the disappearance of Maneb and Zineb with the growth rate of cucumber and tomato under the same conditions (Figs 3 and 4) suggests that the apparent disappearances of Maneb and Zineb are attributed to dilution by the increasing cucumber and tomato weight.

The correlation between the growth of cucumber and tomato with Maneb and Zineb disappearance rate were noticeable, with a correlation coefficient of 0·9992 ($n = 5$). The concentration of ETU, on the other hand, is reduced by the increase in the cucumber weight and tomato weight. The fact that the concentration of ETU at time zero is higher than its concentration at any sampling time (Tables 2 and 3) is expected and is due to the presence of this degradation product in Maneb

Table 3. Residues of Maneb, Zineb and ETU on tomato fruits after various intervals of treatment

Time (day)	Maneb (ppm)		Zineb (ppm)		ETU (ppm)		Change in tomato weight[a]
	Proposed method	Official method[b]	Proposed method	Official method[b]	Proposed method	Official method[b]	
0·00	14·8	14·6	27·9	27·6	71·8	71·3	0·0
0·04	14·2	13·9	26·9	26·6	67·9	67·4	nd
0·25	12·8	13·1	25·2	24·9	56·4	56·0	nd
0·5	11·5	11·3	24·1	24·3	48·8	48·4	0·75
1·0	9·9	9·8	19·7	19·2	41·1	40·5	nd
1·5	9·1	9·2	16·3	16·6	37·4	36·7	0·65
2·0	7·9	7·7	14·7	14·3	32·3	31·5	nd
2·5	7·3	7·0	12·0	11·7	23·4	22·8	0·45
3·0	6·4	6·2	10·8	10·9	20·9	20·3	0·25
3·5	5·9	5·4	8·3	8·1	14·4	13·6	0·15
4·5	4·4	4·2	6·1	5·9	11·7	11·1	0·11
5·5	3·6	3·4	4·2	3·9	10·3	9·5	0·09
6·5	2·3	2·0	2·1	2·0	7·8	7·2	0·03

[a] Ratio between the weight of tomato fruit on application day to the weight on harvesting day.
nd, No determination was made.
[b] From Hylin *et al.* (1978).

formulation and Zineb formulation (Rosenberg & Siltanen, 1979).

As shown in Tables 2 and 3, ETU disappears by dilution on the cucumber surface and the tomato surface at a faster rate than its rate of formation from degradation of Maneb and Zineb. This observation supports the conclusion that Maneb and Zineb were being diluted by the increase in the cucumber weight and tomato weight.

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