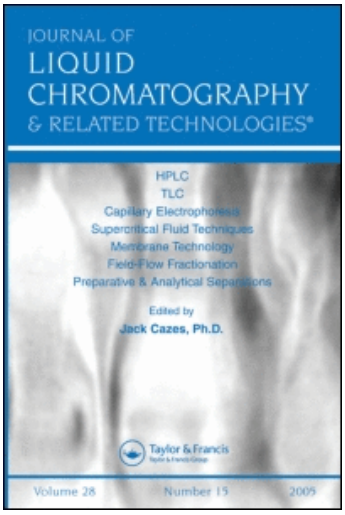

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HPLC STUDY OF MANCOZEB DEGRADATION ON LEAVES

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ABSTRACT

An HPLC procedure was developed for the determination of mancozeb on cucumber leaves after application of mancozeb suspension (ethylenebisdithiocarbamate fungicide). The analytical procedure consisted of a single extraction step with EDTA aqueous solution and sodium salt of ethylenebisdithiocarbamate was eluated from Separon NH_2 column. Mobile phase was 5 % acetonitrile and 45 % methanol in aqueous solution of borate buffer at pH = 7.6 . The level of mancozeb after application on leaves decreased slowly, with a half time 14 days.

INTRODUCTION

Post-harvest diseases of some agriculture products are mainly produced by fungal pathogens. They can cause important economic losses. Thus fungicides treatments are necessary in order to overcome this problem. The fungicides employed are generally toxic and they can present some hazards to public health. Therefore, legal requirements of many countries are increasing, making it necessary to determine fungicides at very low levels.

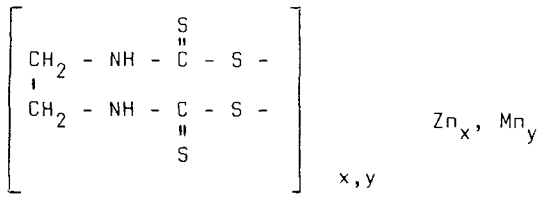

FIGURE 1 Structure of mancozeb

Mancozeb (coordination products of zinc ion and manganous ethylenebisdithiocarbamates - Figure 1) is widely used in agriculture, due to its high fungitoxic activity /1/.

Study of the stability of mancozeb in environmental systems is difficult because many degradation products can be formed. The important decomposition product is ethylenethiourea because this compound is goiterogenic /2/, carcinogenic /3/ and teratogenic /4/.

The methods of analysis of ethylenethiourea in foodstuffs and other substrates were worked out by more authors /5-7/.

Mancozeb is insoluble in water and almost all organic solvents. Therefore, a direct liquid chromatographic determination is impossible. However, mancozeb can be converted into nabam (sodium ethylenebisdithiocarbamate) by the addition of an aqueous EDTA solution. Reversed phase chromatography with an ion-pair reagent in mobile phase can be used /8/.

The present study has used an HPLC method to examine the mancozeb stability on cucumber leaves grown in a greenhouse following application of mancozeb. It has been possible to monitor the levels of mancozeb under controlled conditions and draw up profiles of the changes of mancozeb amount on leaves during 30 days.

EXPERIMENTAL

Chemicals

Solvents for chromatography were of HPLC grade and were purchased from Merck. Mancozeb (wettable powder) was commercial samples. Water was twice distilled.

Extraction of cucumber leaves

Cucumbers were grown in a greenhouse at 20-25 ^{o}C. A separate planting of cucumbers was used for each study. Analyses were carried out on triplicate cucumbers grown and treated under the same conditions. Leaves were harvested by cutting just above the stalk. The leaves (11-15 g) were extracted with 40 ml of an aqueous solution of EDTA (0.1 M) at pH = 9 (borate buffer) for about 5 min in an ultrasonic bath. The extract was filtered and then injected into chromatographic column.

Spraing studies on leaves

Aqueous suspension of mancozeb (0.1 g/50 ml) was sprayed carefully onto individual leaves using a air-powered spray so that the leaves were fully wetted and there was no run-off onto the soil.

Liquid chromatographic system

HPLC separations were carried out using a Waters pump (Model 510), Valco injection valve with a 10 μl loop a diode array detector (Waters Model 910) at 272 nm. The samples were separated using 5 % acetonitrile - 45 % methanol in aqueous solution of borate buffer at pH = 7.6 as the eluent with a flow rate of 0.7 ml/min on Separon NH_2 column (5 μm particle size, 0.32 x 15 cm). The life-time of chromatographic column was about 15 analyses.

RESULT AND DICUSSION

The levels of nabam in extracts were monitored directly by HPLC analysis on a NH_2 column. Care must be taken to optimize HPLC conditions for retention time of nabam sensitivity and selectivity of the separation process. It was found that pH of mobile phase has significant influence on the shape of nabam peak. The pH of mobile phase should be kept at 7.6. The determination of nabam can be carried out with a mobile phase 45 % methanol and 5 % acetonitrile in water. In this system the interference of coextractives with nabam peak was not ob-

served. In Figures 2 and 3 representative chromatograms of extracts as well as treated and untreated samples are shown.

In order to avoid the problem of the mancozeb insolubility, the reaction with EDTA solution was used. The chemical reaction between EDTA and mancozeb is quick and the high yield can be achieved during 5 min (Figure 4). After 20 min nabam is slowly decomposed in standard solutions. Nabam which is formed during the extraction can be more unstable, particularly if they contain plant extract material. Therefore in extraction studies of leaves there have been considerable problems with losses of nabam during the extraction, work-up and assay procedures. It was necessary to estimate the stability of nabam in the leaves ectract and the yield of extraction. A study was therefore carried out in which a single high dose of mancozeb was sprayed onto the leaves and the leaves were harvested and analysed immediately after drying. The dependence of the mean level of nabam in extract on time of extraction is shown in Fig.5. It can be seen that, nabam after 5 min

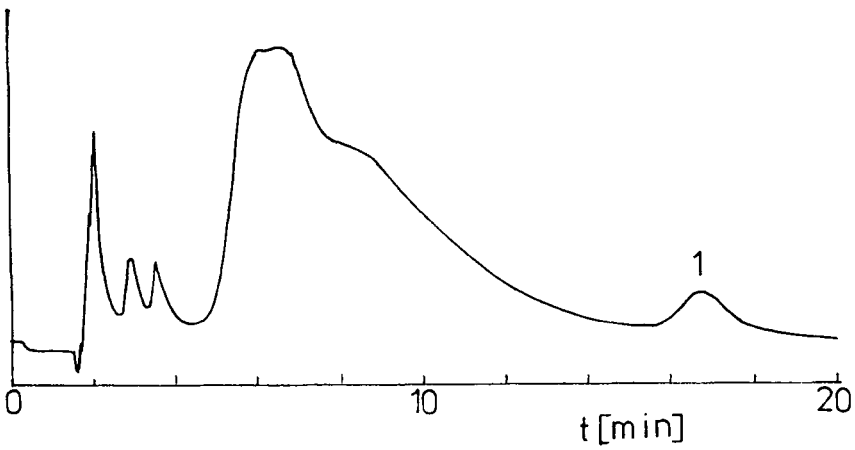

Figure 2. Chromatogram of extract of the treated leaves
Conditions : column Separon NH_2; mobile phase 5 % acetonitrile 45 % methanol in water (pH=7.8) flow rate 0.7 ml/min
Peak : 1 - nabam

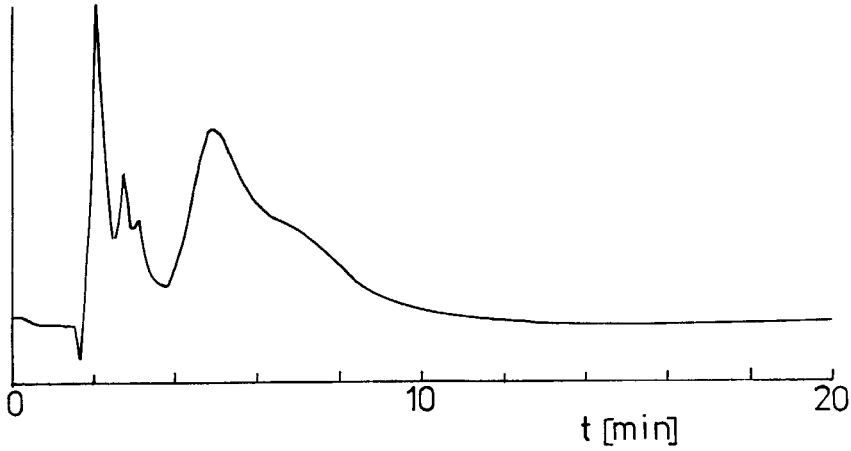

Figure 3. Chromatogram of extract of the untreated leaves For LC conditions, see Figure 2.

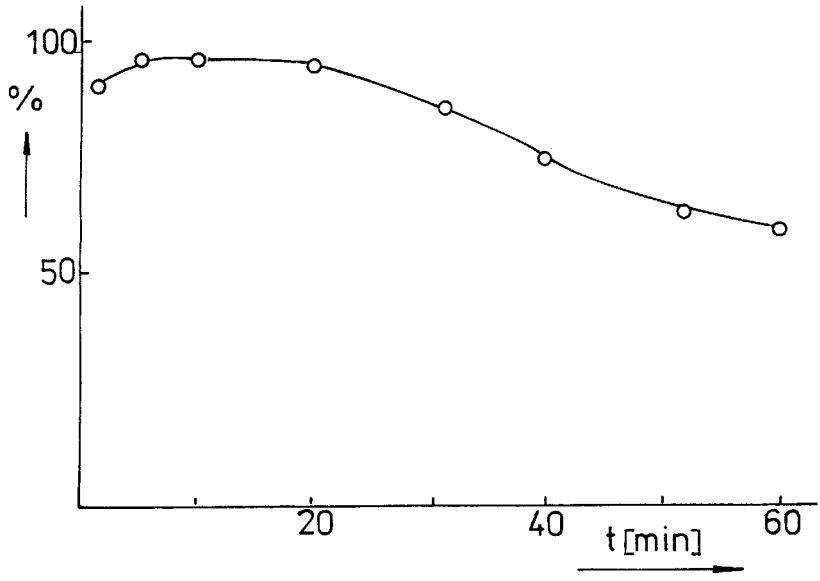

Figure 4. Dependence of reaction yield of nabam on time

is slowly decomposed. This effect may be due to the influence of enzymes released from the plant cells /9/.

In order to determine the life-time of mancozeb on the leaf surface the same levels of aqueous suspension were sprayed onto the leaves cucumbers. Three leaves were harvested at intervals and mancozeb levels determined. After 380/ug/1 g of leaf had been sprayed onto each leaf, the initial concentration (360/ug/1 g of leaf) determined on a surface and analysed 1 hour after application agreed closely with the dose rate applied (Fig.6).

This level of application can be comparible to that expected in commercial operation. There was then a loss of mancozeb with a half-life of about 14 days. For each sample the results from duplicate leaves were similar (see Fig.6). This loss of mancozeb on the leaf surface can be caused by photolysis in the presence of oxygen.

The amount of mancozeb was determined by linear least-squares fitting of the curves of the amount of mancozeb after chemical reaction with EDTA injected against peak area.

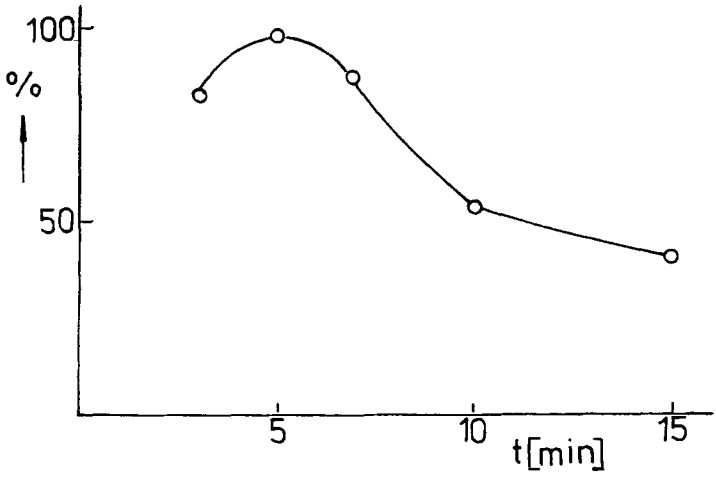

Figure 5. Dependence of nabam mean level in extract on time of extraction

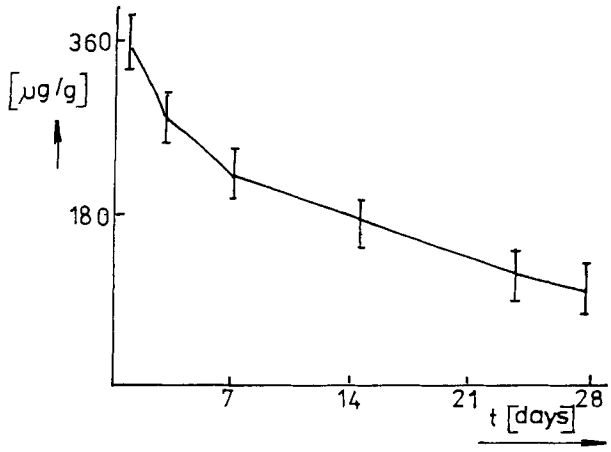

Figure 6. The dependence of mean level of mancozeb on the time after spraying

The concentration of standard solutions were 0.01-0.2 mg/ml of nabam in water, and the calibration graph was linear. The detection limit was about 10 ppm.

REFERENCES

1. N.R.McFarlane, Herbicides and Fungicides, Chemical Society, London, 1977.
2. Graham S.L., Hansen W.H., Bull.Env.Cont.Toxicol.7, 19 (1972).
3. Borge M.U., Weisburger J.H., Weisburger E.K., Rice J.M., Cypher R., J.Nath. Cancer Inst. 49, 583 (1972).
4. Bottomeley P., Hodless R.A., Smart N.A., Residue Rev. 95, 45 (1985)
5. Smith R.M., Madahar K.C., Salt W.G., Pestic.Sci.23, 337 (1988).
6. Nash R.G., Beall M.L., J.Agric.Food Chem.28, 322 (1980).
7. Lehotay J., Brandšteterová E., Oktavec D., J.Liq.Chromatogr. 15, 525 (1992).

8. Irth H., DeJong G.J., Frei R.W., Brinkman U.A.Th., Intern. J.Environ.Anal.Chem. 39, 129 (1990)

9. Ross R.A., Crosby D.G., J.Agric.Food Chem.,21, 335 (1973).

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