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Degradation of Dithane M-45 Residues in Brinjals during Cooking

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The residues of ethylenebisdithiocarbamates (EBDCs) have been found in the treated crops as well as in the adjacent fields (Rosenberg and Siltanen 1979). Although the EBDCs do not pose any serious problem directly, the presence of ethylenethiourea, a degradation product of the EBDCs in the harvested crop is of great concern due to its known harmful effects (Khera 1987). It has been reported that the plant products containing the relatively less toxic EBDCs residues may yield significant amounts of ETU during cooking thus adding to the ETU residues further. In the present studies an assessment has been made regarding the amount of both dithane M-45 and ETU present on harvest and after cooking in brinjal fruits (Solanum melongena).

MATERIAL AND METHODS

Two types of fruits-fruits freshly treated with ^{14}C labelled mancozeb (6 in number) and fruits with weathered residues of ^{14}C mancozeb treated 15 days earlier (12 in number) were used in this study. The experiment with weathered residues was conducted in November 1987 and was repeated in October 1988. ^{14}C mancozeb was extracted with freshly distilled ethyl acetate four times to remove impurities, such as ETU and other metabolites. The purified mancozeb gave only one spot on thin layer chromatography (TLC). In the case of freshly treated fruits an aqueous suspension of ^{14}C mancozeb (500 µl) was applied to each fruit. In order to obtain field weathered samples, the brinjal fruits still attached to plants were treated with aqueous suspension of ^{14}C mancozeb which contained 2 mg mancozeb and 1.3 µCi ^{14}C activity in case of November 1987 experiment. However in October 1988 experiment the amount of ^{14}C activity was 1.09 µCi and 1.81 mg mancozeb. Half of the weathered samples (6 in number) were washed with running tap water for 3 minutes. Samples from each category i.e. freshly treated, weathered unwashed, and weathered and washed, in triplicate were cooked in water for 25 minutes at 92-97

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°C. The remaining samples formed the controls. Representative samples from each group (70 per cent w/w of sample) were extracted first with methanol and then with chloroform in a high speed blender. 200 ml methanol and 100 ml choloform were used for 100gm samples. The homogenized samples were filtered with suction through a 1.5 cm bed of celite 545 sandwiched between two Whatman no.1 filter paper and placed over a 15 cm buchner funnel. The residues left were re-extracted with 0.4 M ethylenediamine tetra acetic acid (EDTA) for the parent compound as well as some possible conjugates which might have escaped the previous extraction. Control samples in triplicate from each group were also extracted in the same way.

The filtrate obtained after methanol and chloroform extraction was washed with 200ml water. The chloroform layer was separated and rewashed with 100 ml water and the aqueous layers combined. The methanol:water portion obtained thus was partitioned again with chloroform, and the chloroform layer obtained was washed with water. All the aqueous portions were combined. One ml of 50 per cent NaOH was added to the aqueous extract and the volume was reduced to about 5 ml under vacuum at 60 °C. The pH of the extract was adjusted to 7 by adding 6N HCl. The volume was adjusted to 10 ml with water.

The fungicide residues in the samples were determined by the evolution of carbon disulfide resulting from acid hydrolysis and measured by spectrophotometric method (Keppel 1969). Aliquots of the intact samples (before extraction) were used for this purpose. For the analysis of degradation products the extract obtained after clean up and reduction was subjected to thin layer chromatography using precoated silica gel 1B2 flexible sheets for TLC obtained from J.T.Baker Chemical Co.(West Germany). The solvent system used was chloroform:butanol:methanol:water (100:5:1:0.5,v/v). A mixture of standards was also applied on each sheet along with the samples. Iodine vapours were used to visualise the spots on TLC. Identification of different metabolites was made by using the rate of flow (Rf) of the standards and those mentioned in the literature. The spots corresponding to the various compounds were cut and taken into scintillation vials for radioassay on a Packard Model 2000 CA Tri-carb Liquid Scintillation Analyser with automatic quench correction facility. Appropriate aliquots from the filtrate obtained after the EDTA extraction were also analysed for radioactivity and the counts (dpm) corresponding to this were added to the counts corresponding to the origin of the TLC sheets representing the parent compound.

RESULTS AND DISCUSSION

In the freshly treated fruits only ETU could be detected in addition to mancozeb. In the control samples 11.4 per cent of the radioactivity was in the

form of ETU which increased to 45.6 per cent as a result of cooking. This showed the degradation of the parent fungicide at high temperature of cooking in water. Watts et al (1974) reported that about 20 per cent ETU was formed as a result of boiling fortified spinach with mancozeb. The presence of 11.4 per cent of ETU in uncooked samples may be due to decomposition during the extraction procedures (Pazmino et al 1989). In case of weathered samples ethylenethiuram disulfide, ethylenethiuram monosulfide and certain unknown compounds were found to be present besides ETU and mancozeb. Tables 1 and 2 present the data on the residues of mancozeb recovered 15 days after treatment and then washing and cooking. In the Nov 1987 experiment the controls had 47.5 per cent of the mancozeb (10.98 µg/gm fresh weight) applied after 15 days of treatment. As a result of washing the mancozeb residues were reduced to about 22.3 per cent of the original (5.53 µg/gm fresh weight). In Oct 1988 experiment 37.5 per cent of the mancozeb (10.64 µg/g fresh weight) applied was recovered 15 days after treatment. Washing reduced it to 17.8 per cent of the applied. Cheah (1985) reported that in tomatoes the mancozeb residues were reduced to 0.58 mg/kg CS_2 equivalent from 3.8 mg/kg CS_2 equivalent on washing. It is thus seen that the surface washing of vegetables even for a short time removes a large proportion of the EBDC residues and the practice followed normally is indeed very beneficial in reducing fungicide residues. Mancozeb residues further reduced to more than 1/3rd due to cooking. Only 1.85 µg/gm (fresh weight) of mancozeb was recovered in washed and cooked samples. Cheah (1985) also observed the removal of 12-79 per cent of mancozeb residues from tomato on boiling for 10 minutes.

The data on the amounts of the degradation products is shown in table 3 and is based on the average values obtained from the two experiment. The amount of ETU in the washed samples was 216.5 µg/g as compared to 1550 µg/g in case of unwashed samples. Cooking reduced the amount of ETU from 1550 µg/g to 342.9 µg/g in case of unwashed samples while in case of washed samples the amount of ETU decreased from 216.5 µg/g to 181.4 on cooking. However, increase in amounts of ethylenethiuram disulfide (ETD), ethylenethiuram monosulfide (ETM) and unidentified metabolites were observed on cooking of both washed and unwashed samples. Ross et al (1978) reported about 34 per cent conversion of mancozeb to ETU in apples as a result of boiling in water. Kolankaya et al (1989) reported total disappearance of both EBDC and ETU from mancozeb treated fruits on boiling.

Table 1. Mancozeb residues recovered from brinjals treated with mancozeb and subsequently washed and cooked. (n = 3)

Unwashed				Washed			
Control		Cooked		Control		Cooked	
Originally applied	Recovered	Originally applied	Recovered	Originally applied	Recovered	Originally applied	Recovered
23.12±1.2	10.98±0.41	23.12±1.2	2.8±0.001	24.80±1.1	5.53±0.12	24.80±1.1	1.85±0.01*
166.8±8.7	79.25±2.9	166.8±8.7	20.18±0.01	178.9±7.9	39.92±0.87	178.9±7.9	13.35±0.07**
	47.5		12.1		22.3		7.5***

Each brinjal was treated with 2.0 mg Dithane M-45 (extracted with ethyl acetate 4 times) and 1.3 µCi ^{14}C mancozeb,Sp.act.1430 dpm/µg.The brinjals were plucked and processed 15 days later. The brinjals were treated on November 17,1987.Mancozeb estimated by CS evolution method.

* mg residues per kg brinjal fresh weight
** mg residues per kg brinjal dry weight.
*** per cent recovered.

Table 2. Mancozeb residues recovered from brinjals treated with mancozeb and subsequently washed and cooked (n=3).

Unwashed				Washed			
Control		Cooked		Control		Cooked	
Originally applied	Recovered	Originally applied	Recovered	Originally applied	Recovered	Originally applied	Recovered
28.39±2.2	10.64±0.35	30.82±2.55	4.9±0.39	31.52±4.78	5.61±0.84	31.21±1.65	1.86±0.13*
205.04±18.1	76.85±2.52	222.20±19.3	35.33±2.83	227.87±24.1	40.56±5.98	220.5±11.72	13.41±0.82**
	37.48±1.67		15.9±0.15		17.8±0.09		5.96±0.2***

Each brinjal was treated with 1.81 mg Dithane M-45 (extracted with ethyl acetate 4 times) and 1.09 µCi ^{14}C mancozeb,Sp.act.1325 dpm/µg.The brinjals were plucked and processed 15 days later.The brinjals were treated on October 5,1988.Mancozeb estimated by CS_2 evolution method.
* mg residues per kg brinjal fresh weight
** mg residues per kg brinjal dry weight.
*** per cent recovered.

Table 3. Different metabolites recovered from brinjals 15 days after treatment with ^{14}C mancozeb and subsequent washing and cooking (n=6).

Treatment		ETU	ETD	ETM
Unwashed				
	Control	1550.3 ±172.3 (9930.1±1098.3	6.4 ±0.1 (57.8±12.8)	9.1* ±0.9 (63.4±9.1)
	Cooked	342.9 ±68.6 (2477.2±493.9)	12.9 ±2.6 (95.8±19.3)	13.6 ±1.8 (99.8±13.6)
Washed				
	Control	216.5 ±27.4 (1565.6±193.6)	11.6 ±3.2 (83.6±25.7)	13.6 ±2.7 (81.6±22.7)
	Cooked	181.4 ±91.4 (690.6±141.7)	4.5 ±1.2 (32.2±6.4)	4.5 ±1.3 (40.8±9.1)

* mg metabolite per kg brinjal fresh weight.
Values in parentheses are on dry weight basis.

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