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Persistence, Metabolism, and Movement of Ethylenethiourea in Eggplant (*Solanum melongena* L.) under Subtropical Conditions

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In the subtropical region vegetables and cereal crops are most vulnerable to the fungal pathogens because of the warm and humid climate. Of the various fungicides available for controlling these pathogens ethylenebis-dithiocarbamates (EBDCs) are most effective and widely used. EBDCs once a boon to farmers have recently been a subject of controversy. Although these fungicides are themselves comparatively safe because of their low mammalian toxicity and very low persistence, the presence of 2-imidazolidinethione / ethylenethiourea (ETU), a well known neuroteratogen and carcinogen in rodents (Ulland et al 1972, Khera 1987), in commercial formulations and its formation during the storage and metabolism of EBDCs in plants (Onley et al 1977, Ripley and Simpson 1977, Rosenberg and Siltanen 1979) has caused great concern. There is also ample evidence that higher amounts of ETU are likely to be formed in tropical and subtropical regions than in temperate climate, since EBDCs are known to be unstable in the presence of moisture and ETU yields increase with elevated temperature (IAEA- TECDOC, 1989). ETU may be formed on the plant surface following the foliar application of EBDCs and the former being water soluble is likely to move to other parts of the plants and persists there for sometime (Hoagland and Frear, 1976). Egg-plants (Solanum melongena L.) are most often treated with EBDC fungicides to protect them against various fungal pathogens. Our previous finding (Kumar and Agarwal, 1990) on the presence of significant amounts of ETU in egg-plants treated with mancozeb, an EBDC, necessitated the present study of the fate of ETU itself in egg-plants (brinjal plants).

MATERIALS AND METHODS

^{14}C-ETU used in the present experiments was synthesised in the laboratory using [U-^{14}C]-ethylene diamine

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dihydrochloride (Kumar, 1989). The final product was found to be free of any impurities (99% pure) as revealed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The specific activity of ^{14}C-ETU was 0.386 µCi/mg.

Twelve leaves on each of the 20 potted egg-plants were randomly selected at different levels on the plant and were suitably marked. These leaves were treated with an aqueous solution of ^{14}C-ETU, so that each plant received 3.34 µCi ^{14}C-ETU in a total volume of 1.25 ml equally distributed on 12 leaves. Zero time samples of the treated leaves (12 leaves each from 3 randomly selected plants) were taken immediately after application. Subsequently all the treated and untreated leaves and fruits of the individual plants were taken as samples. Each sample consisted of 3 plants selected at random and taken on 3,7,14,28 and 56 days after treatment. The samples (5-10 gm) were cut into small pieces and homogenised in a high speed blender using 100 ml methanol and filtered. The extraction was repeated thrice and the extracts combined. 100 ml water was added to this combined extract and the volume reduced to about 75 ml *in vacuo*. The extracts were cleaned up with hexane as described by Rhodes (1977). The aqueous portion was reduced to 5.0 ml *in vacuo*. Aliquots ranging from 50-250 µl were applied on two TLC plates of glass coated with 250 µm thick silica gel G layer (E. Merck, Germany) alongwith the standards. The plates were run separately using two different solvent systems, I. Chloroform : 1-Butanol : Methanol : Water (100:5:1:0.5, v/v) and II. Ethyl acetate : Ammonia (25%) : Water (15:1:1, v/v). The TLC plates were exposed to iodine vapour to visualise the spots. The spots cochromatographing with the standards were scrapped and radioassayed on a Packard Model 2000 CA Tri-Carb Liquid Scintillation Analyser with automatic quench correction facility. The scintillation cocktail used was Aquasol Universal LSC cocktail (NEN Research Products, USA). 10 µl aliquots were also radioassayed for total ^{14}C-activity.

Some of the samples were extracted and cleaned up as above and were subjected to further clean up for HPLC using Extrelut column (E. Merck, Germany) and dichloromethane (Kobayaski *et al.*, 1986). The effluent was evaporated to dryness *in vacuo* and dissolved in 2 ml of water:methanol (95:5,v/v). A 50 ul aliquot of the cleaned up extract was injected onto a Shimadzu LC-4A HPLC with a variable UV detector using Zorbax-ODS RP column (15 cm L x 4.6 mm i.d.) at 240 nm and 0.16 aufs. The column was eluted with water and methanol (95:5,v/v) at the flow rate of 0.8 ml/min. Under these conditions ETU eluted 3.7 minutes after injection.

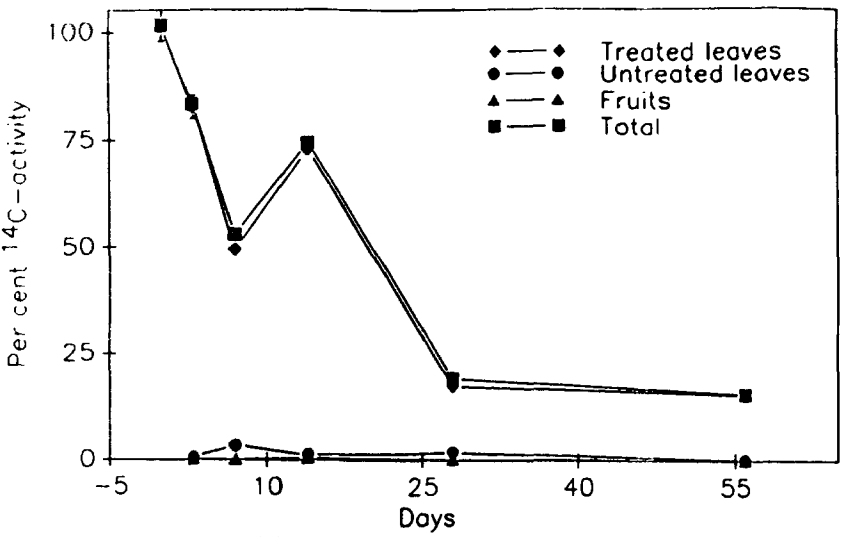

Figure 1. Per cent ^{14}C-activity recovered when ^{14}C-ETU was applied to 12 leaves of each plant with respect to time.

Fractions corresponding to ETU were collected and radioassayed to detect the presence of ^{14}C-ETU in the samples.

RESULTS AND DISCUSSION

ETU being a water soluble compound, may enter the plant tissues and may be translocated to other parts as well as the soil. Thus studies were undertaken to investigate the persistence of ^{14}C-ETU, its metabolism and translocation in egg-plants. The importance of such studies can be better understood by the fact that the data on the residues of EBDCs and ETU is very scanty from the subtropical region. The MRL value for EBDCs in India has been fixed at 3 ppm CS_2 equivalent for fruits and tomatoes. In the present experiments, the total recovery of ^{14}C-activity at different time intervals in different parts of egg-plants is shown in Figure 1. Bulk of the ^{14}C-activity was from the treated leaves and it decreased to about 15 per cent, 56 days after treatment. Significant amounts of ^{14}C-activity ranging from 0.11 to 3.4 per cent were detected in untreated leaves. The fruit samples had the lowest amount of ^{14}C-activity ranging from 0.05 to 0.44 per cent. Generally in treated leaves a lot of ^{14}C-activity was present at the origin of the TLC plate. Among the identified metabolites ethyleneurea (EU) was the predominant one followed by hydantoin in the treated leaves. Three more spots were obtained which could not be identified at this time. The amount of ETU and EU (µg/gm dry weight of the tissue) in plants at different periods are shown in Table 1. About 1/3rd to 1/4th of the ETU was

Table 1. Total ETU and EU recovered from different parts of brinjal after application of ^{14}C-ETU to 12 leaves of each plant with respect to time*.

Days after treatment		Treated leaves (µg/gm dry weight**)	Untreated leaves	Fruits	Total
0	ETU	5726 ±79.36	-	-	5726
	EU	27.7 ±0.91	-	-	27.7
3	ETU	2424 ±86.88	4.97 ±0.68	0.47 ±0.09	2429
	EU	1223 ±61.35	4.37 ±0.86	-	1227
7	ETU	2883 ±37.6	30.1 ±0.29	0.65 ±0.13	2914
	EU	49.99 ±1.25	15.87 ±1.08	1.003 ±0.19	66.86
14	ETU	2408 ±55.06	4.62 ±0.11	3.31 ±0.26	2416
	EU	951 ±26.7	13.23 ±0.78	3.154 ±0.19	967
28	ETU	21.96 ±0.70	8.24 ±0.78	0.67 ±0.19	30.9
	EU	179.8 ±2.77	13.21 ±2.36	2.25 ±0.32	195.3
56	ETU	5.5 ±1.61	1.23 ±0.68	0.69 ±0.12	7.4
	EU	124 ±13.56	0.77 ±0.15	1.21 ±0.29	126.0

Twelve leaves on each plant were treated with aqueous solution of 3.34 µCi ^{14}C-ETU (8.65mg).
* All leaves and fruits of all the three plants making the sample were analysed.
** Dry weight was calculated on the basis of water content estimated in the control leaf samples of the day corresponding to sampling by gravimetric method.

recovered as such upto 14 days after treatment which subsequently reduced to about 0.06 per cent after 56

days. Similarly in fruits also the proportion of ETU varied during the entire period with a maximum value of 3.31 µg/gm on 14 days after treatment and a minimum value of 0.47 µg/gm 3 days after the treatment. The rapid growth of fruits as compared to leaves may also be one of the reasons of the decline in the concentration of ETU besides it degradation to EU. The ETU detected in the whole plant ranged between 2416 µg/gm to 2914 µg/gm (about 28 to 34 per cent of the applied dose) upto 14 days after treatment. A sharp decline to 30.9 µg/gm and then to 7.4 µg/gm was observed on 28 and 56 days after treatment. HPLC analyses followed by radioassay of the fractions corresponding to ETU showed the presence of ^{14}C-ETU (originally applied to leaves) in all the samples.

Rhodes (1977) observed that about 28 per cent of the applied ^{14}C-activity was obtained 10 days after the treatment of tomato foliage and stems with ^{14}C-ETU. The unchanged ETU, however, was only about 0.45 per cent of the amount applied at that time. In the case of bean foliage and stems also about 29 per cent of the original radioactivity applied could be obtained 10 days after treatment with only 0.5 per cent being intact ETU.

In the present study significant amounts of EU were detected in the plant, bulk being present in the treated leaves. The amount of EU in case of treated leaves was variable during the entire period. The initial amount of 27.7 µg/gm at zero time increased up to 1223 µg/gm on the 3rd day which then came down sharply to 49.99 µg/gm on 7th day. In case of untreated leaves, however, the value remained almost constant during the period from 7 days after treatment to 28 days after treatment. Relatively low values of 4.37 µg/gm and 0.77 µg/gm were observed on 3 days and 56 days after treatment. In fruits the EU content increased initially in 14 day samples and gradually declined thereafter to 1.21 µg/gm in 56 day samples. The percentages of ETU and its metabolites as determined by TLC and radioactivity estimations of the total recovered dose are presented in Table 2. The percentage of ETU was high in the treated leaves initially but it ultimately declined to 0.105 per cent in 56 day samples. In the untreated leaves the maximum radioactivity was present at the origin of the TLC plates. ETU, EU and hydantoin were the major compounds identified in the untreated leaves. The metabolites detected and their proportions in the fruits were somewhat similar to the untreated leaves.

The data on the dissipation of ^{14}C-ETU from the leaves were subjected to kinetic analysis (Figure 2). It was

Table 2. Per cent ETU and its metabolites detected in different parts of brinjal plants after tratment with ^{14}C-ETU.

Days	Sample	Origin*	EU	Hydantoin	ETU	IV*	V*	VI*
0	TL	1.76	0.57	-	99.24	-	-	-
3	TL	12.32	25.14	2.98	42.01	0.17	0.02	0.008
	UL	0.32	0.064	0.029	0.061	0.029	0.026	0.037
	F	0.022	-	0.01	0.016	-	0.032	-
7	TL	11.53	0.792	9.009	27.32	0.396	0.297	0.198
	UL	2.336	0.333	0.122	0.53	0.044	0.024	0.024
	F	0.031	0.009	0.003	0.005	0.003	0.004	0.005
14	TL	25.34	14.67	1.016	31.36	0.189	0.087	0.08
	UL	0.794	0.272	0.031	0.08	0.014	0.004	0.003
	F	0.272	0.05	0.031	0.044	0.015	0.017	0.01
28	TL	12.75	3.724	0.505	0.383	0.017	0.017	-
	UL	1.081	0.262	0.155	0.138	0.115	0.042	0.027
	F	0.077	0.027	0.011	0.007	0.008	0.009	0.012
56	TL	11.35	2.82	0.395	0.105	0.012	0.038	0.454
	UL	0.043	0.007	0.009	0.01	0.005	0.003	0.033
	F	0.022	0.007	0.003	0.003	0.003	0.006	0.005

TL= Treated leaves, UL= Untreated leaves, F= Fruits.
* Unknown

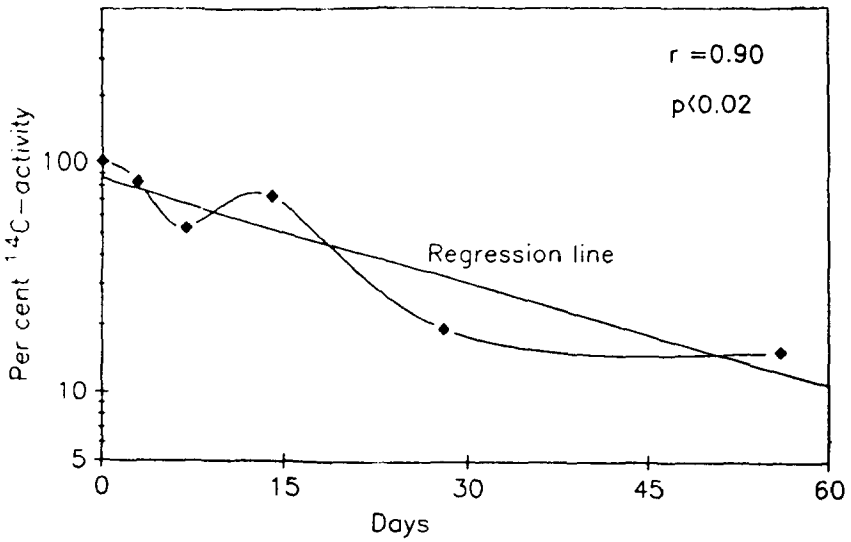

Figure 2. Dissipation of ^{14}C-ETU applied to the leaves of egg plants.

found that ^{14}C-ETU dissipated rapidly from the leaves. However, the rate of dissappearance was slower from leaves with a lower rate constant of 0.03475 as compared to fruits (Kumar, 1989). The dissipation followed the first order kinetic reaction. The time taken for the dissappearance of half of the amount present at a particular time (T 1/2) was 19.94 days. Hoagland and Frear (1976) reported the degradation of ^{14}C-ETU in lettuce, pepper and tomato seedlings treated with ^{14}C-ETU, however, 20-30 per cent of ^{14}C-ETU was still present after about 12 days. They found that the movement of ^{14}C-activity from the site of injection of stems was relatively low, and acropetal and basipetal movement of ^{14}C-ETU and ^{14}C-labelled products was very limited. In the present experiment ^{14}C-ETU was applied on the leaves with the help of a microsyringe. Thus the presence of ^{14}C-activity in other parts may be related to the movement of ^{14}C-ETU and not as a result of degradation of EBDCs on the fruits or other parts received during foliar spray. The present investigations reveal that ETU is translocated from one part of the egg-plant to other parts and also it degrades fast under the influence of high temperatures of subtropical region. EU which is formed from ETU appears to be more stable. Thus the problem of ETU residues in vegetables may not to be of so much concern as it gets converted rapidly into EU, a relatively harmless compound.

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REFERENCES

Hoagland RE and Frear DS (1976) Behaviour and fate of ethylenethiourea in plants. J Agric Food Chem 24:129-133

IAEA-TECDOC-554 (1989) Radiotracer studies of fungicide residues in food plants: A technical document issued by the International Atomic Energy Agency, Vienna, p 111

Khera KS (1987) Ethylenethiourea: a review of teratogenicity and distribution studies and an assessment of reproduction risk. CRC Critical Reviews in Toxicology 18: 129-139

Kobayaski H, Matano O, Goto S (1986) An improved method for residue analysis of ethylenethiourea in vegetables by high-performance liquid chromatography. J Pestic Sci 11:81-84

Kumar U (1989) Persistence and metabolism of ethylene-bisdithiocarbamates in brinjal plants and the effect of storage, cooking and solar radiation on their degradation. PhD Thesis, University of Delhi.

Kumar U, Agarwal HC (1990) Persistence of ^{14}C-Maneb and mancozeb on egg-plants (Solanum melongena) under sub-tropical conditions. Pestic Res J 2: 83-90

Onley JH, Giuffrida L, Ives NF, Watts RR, Storherr RW (1977) Gas-liquid chromatography and liquid chromatography of ethylenethiourea in fresh vegetable crops, fruits, milk, and cooked products. J Assoc Off Anal Chem 60:1105-1110

Rhodes RC (1977) Studies with manganese [^{14}C]ethylene-bis (dithiocarbamate)([^{14}C]maneb) fungicide and [^{14}C]-ethylenethiourea ([^{14}C]ETU) in plants, soil and water. J Agric Food Chem 25: 528-533

Ripley BD, Simpson CM (1977) Residues of zineb and ethylenethiourea in orchard treated pears and commercial pear products. Pestic Sci 8: 487-491.

Rosenberg C, Siltanen H (1979) Residues of mancozeb and ethylenethiourea in grain samples. Bull Environ Contam Toxicol 22:475-478

Ulland BM, Weisburger JH, Weisburger EK, Rice JM, Cypher R (1972) Thyroid cancer in rats from ethylene-thiourea intake. J Natl Can Inst 49:483-484

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