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STANDARDISATION OF PROCEDURE FOR RESIDUE ANALYSIS OF 2, 4-D IN SOIL

Colorimetry is one of the earliest instrumental techniques for the estimation of 2,4-D residues and even today it continues to be a useful method wherever costlier modern facilities like gas chromatograph are not readily available. Freed (1948) developed a qualitative method in which 2, 4-D was heated with chromotropic acid in concentrated sulphuric acid at 150 °C and the development of wine purple colour was taken as a measure of 2, 4-D concentration. Subsequent modifications of Freed's procedure by Marquardt and Luce (1951 and 1955) and Bandurski (1947) helped in the quantification of 2, 4-D residues in milk, seeds, grains and soil. All these procedures involve large number of extraction steps with high volumes of organic solvents for eliminating the analytical interferences from other compounds. It is necessary to simplify the extraction and separation procedures for saving time and volume of costly chemicals, without reducing the recovery percentage. With these views, an attempt was made to modify the available colorimetric techniques for the detection of 2, 4-D residues in soil.

Soil samples collected from the kole lands of Thrissur district were pooled, processed and samples of 10 g each were fortified at the level of 1 ppm with technical grade 2, 4-D acid dissolved in methanol. Fortified samples were kept for a period of half an hour and shaken with solvents (as indicated in Table 1) at soil:solvent ratios of 1:2 and 1:4 for a period of 30 min. The filtrate was shaken with 100 ml $1N$ NaOH, 15 ml conc. HC1 and 50 ml diethyl ether in a 250 ml separating funnel. The ether portion was separated and the aqueous layer was again shaken with 50 ml diethyl ether. The ether portions were pooled and extracted twice with 25 ml of buffer solution (25 g $Na_2HPO_4.12H_2O$ and 10 g $NaH_2PO_4.H_2O$ in 1 litre of distilled water). The pooled buffer separate was shaken with 1 ml conc. HC1 and 10 ml carbon tetrachloride. The aqueous layer was separated and again shaken with 10 ml carbon tetrachloride. The pooled carbon tetrachloride layer was kept for evaporation and the residue was treated with 3 ml chromotropic acid reagent (0.4 g chromotropic acid in 100 ml of conc. H_2SO_4) and kept in an oven at 135 °C for 20 min. The wine purple coloured complex obtained was cooled, diluted and made up to 50 ml with distilled water. Absorbance of the coloured complex was read on a visible spectrophotometer at 565 nm against a reagent blank set at 100 per cent transmission. A standard absorption curve for 2, 4-D acid was prepared by taking 2, 4-D standards viz., 0.00, 0.05, 0.10, 0.20, 0.40, 0.80, 1.00 and 2.00 ppm in methanol and developing the colour using the same procedure as used for soil sample.

In order to confirm the reproducibility of the method, 15 soil samples (five each from kole, Kuttanad and Palakkad) were fortified with 2, 4-D acid at three levels viz., 0.50, 1.00 and

Table 1. Recovery of 2, 4-D acid from soil by different solvent systems

Sl. No.	Solvent systems	2, 4-D acid added, mg	Absorbance		2, 4-D acid in the extract, mg		Recovery %	
			1:2	1:4	1:2	1:4	1:2	1:4
1	Acetonitrile	0.01	0.009	0.009	0.0050	0.0050	50.00	50.00
2	Acetic acid	0.01	0.008	0.009	0.0045	0.0050	45.00	50.00
3	Benzene	0.01	0.003	0.004	0.0017	0.0022	17.33	22.33
4	Acetonitrile: distilled water: glacial acetic acid (80:20:2.5)	0.01	0.016	0.017	0.0088	0.0094	88.88	94.44
5	Chloroform: ether: acetic acid (50:50:1)	0.01	0.008	0.008	0.0045	0.0045	45.00	45.00
6	1% NaOH (0.25*M*)	0.01	0.104	0.104	0.0560	0.0580	Turbidity interference	
7	4% NaOH(1.0*M*)	0.01	0.108	0.108	0.0570	0.0600		
	CD (0.05)						12.44	9.31

2.00 ppm and the recovery of 2, 4-D from the soils was estimated by adopting the same procedure.

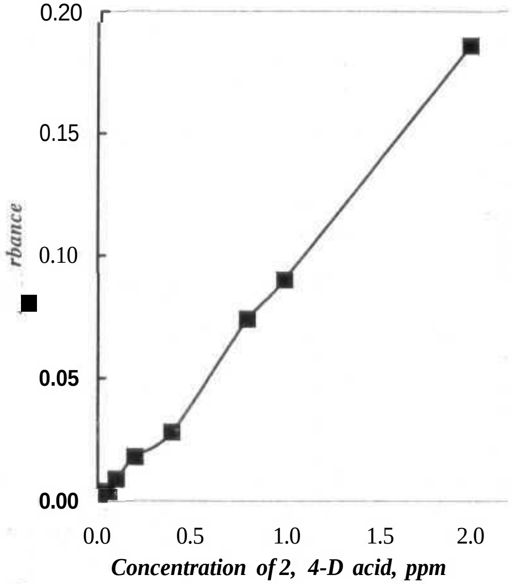

Fig 1. Reference curve for the estimation of 2, 4-D residues in soils by the modified method

Calibration curve obtained by plotting seven concentrations of 2, 4-D on the X axis and their absorbance values on the Y axis was linear (Fig. 1) and it indicated the sensitivity of the method to detect 2, 4-D residues in soil as low as 0.05 ppm. Maximum recovery of 2, 4-D from soil was obtained (94.44 %) when the soil was shaken with acetonitrile : distilled water : glacial acetic acid (80:20:2.5) at 1:4 soil solution ratio with an equilibration period of 30 min (Table 1).

Among the extractions tried, sodium hydroxide was least suited for 2, 4-D estimation. Both the concentrations of NaOH (1% and 4%) brought into solution co-extractives to a great extent. The turbidity and dark colour of the filtrate obtained in these two treatments showed the necessity for heavy clean up. The rest of the four solvents recorded lower recovery percentage. Among the two soil-solvent ratios, 1:4 ratio was superior to 1:2 as evidenced from their higher absorbance readings.

The procedure developed here is an integration and modification of the various procedures used in the extraction of 2, 4-D from milk, grains and soils (Marquardt and Luce, 1951; Smith, 1978). The modifications were mainly made in the time of shaking and the volume of diethyl ether used for separation.

Aqueous acidic-acetonitrile (acetonitrile : distilled water : glacial acetic acid; 80:20:2.5) had been shown to be an efficient extractant of 2, 4-D from soils (Smith and Muir, 1980). In the proposed extraction procedure, the time of shaking was reduced to 30 min. as against 1 h in the original procedure of Smith (1978). The procedure of separation of 2, 4-D from milk (Marquardt and Luce, 1951) was used here with little modifications. Considerable saving in the expensive organic solvent was achieved by changing the volume of diethyl ether from 200 ml to 100 ml.

Table 2. Recovery of 2, 4-D acid from soil at different levels of fortification

Soil sample No.	Recovery of 2, 4-D at (ppm)		
	0.5	1.00	2.00
Palakkad			
S_1	0.39	0.65	1.72
S_2	0.42	0.87	1.44
S_3	0.46	0.87	1.20
S_4	0.50	0.80	1.67
S_5	0.37	0.52	1.22
Kole			
S_6	0.20	0.87	0.94
S_7	0.45	0.71	0.78
S_8	0.50	0.89	1.51
S_9	0.50	0.77	1.53
S_{10}	0.49	0.45	1.22
Kuttanad			
S_{11}	0.45	0.86	1.45
S_{12}	0.49	1.00	1.68
S_{13}	0.45	0.87	1.77
S_{14}	0.43	0.98	1.26
S_{15}	0.50	0.72	1.91

Addition of 1*N* sodium hydroxide and concentrated hydrochloric acid followed by extraction with diethyl ether removed much of the co-extractives. The buffered extraction soludon was used to remove acidic materials other than 2, 4-D from the ether solution. By adopting this procedure, the recovery of 2, 4-D from the 15 soils at fortification levels of 0.50, 1.00 and 2.00 ppm was satisfactory (Table 2). The results suggested that the procedure developed is suitable for the estimation of 2, 4-D residues irrespective of the soil types.

College of Horticulture
Trichur 680 656, Kerala, India

K. M. Durga Devi
C. T. Abraham, N. P. Chinnamma

REFERENCES

Bandurski, R.S. 1947. Spectrophotometric method for the determination of 2, 4-D. *Bot. Gaz.* 108: 446

Freed V.H. 1948. Qualitative reaction for 2, 4-dichlorophenoxy acetic acid. *Science* 107: 98

Marquardt, R.P. and Luce, E.N. 1951. Determination of small amounts of 2, 4-D in milk. *Anal. Chem.* 23: 1484

Marquardt, R.P. and Luce, E.N. 1955. Determination of 2, 4-D in grain and seed. *J. Agric. Food Chem.* 3: 51-53

Smith A.E. 1978. Relative persistence of di- and trichlorophenoxyalkanoic acid herbicides in Saskatchewan soils. *Weed Res.* 18: 275-279

Smith, A.E. and Muir, D.C.G. 1980. Determination of extractable and nonextractable radioactivity from Prairie soils treated with carboxyl and ring labeled [^{14}C] 2, 4-D. *Weed Res.* 20: 123-129