

## BIOASSAY FOR DETECTING CERTAIN HERBICIDE RESIDUES IN SOILS

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Bioassay using cucumber growth was utilised to detect the residues of some common herbicides in soil. The experiment was conducted under pot culture conditions in alluvial (entisol), black (vertisol) and red (Alfisol) soils. Atrazine, oxadiazon, oxyfluorfen and terbutryn inhibited the germination, plant height and dry matter production of cucumber. The red soil exhibited acute toxicity even at lower concentration and the LD 50 of these herbicides were lower than the alluvial and black soils. The detection of residues in the unknown soil can be easily computed from the derived quadratic equations.

The period during which a herbicide remains biologically active in the soil is important in determining both its effectiveness and usefulness. Persistence beyond the critical period of control leads to residue problems in succeeding crops. Ideally a herbicide should retain activity long enough to provide satisfactory weed control, and degrade to innocuous products before it is necessary to apply it again (Hiltbold, 1974). The herbicides that are used for various crops may persist longer than the growing period of the treated crop. Detection of the presence of a herbicide can be done by bioassay which measure the biological response of a living plant to a herbicide and to quantify its concentration in a substrate. A major advantage of the bioassay is the assurance that the phytotoxic activity of the herbicide molecule is being measured. A secondary advantage is that it is not generally necessary to extract the herbicide from the substrate. Bioassay procedures are usually more economical, less difficult to perform and do not require as

much expensive equipments as chemical and physical analytical methods (Santelmann, 1971).

There are various procedures for conducting herbicide bioassays. Parker (1966) and Reid and Hurlt (1969) suggested sensitive root and shoot bioassays for herbicides and growth regulators. Da silva *et al.* (1976) recommended cotyledon disc bioassay for certain herbicides. Eshel and Prendeville (1967), Jacques and Harvey (1979) are some of workers who did bioassay for herbicides like paraquat and dinitroanilines. In this study the cucumber (*cucumis sativa* L.) growth bioassay was used to determine the persistence of some common herbicides that are invariably used for field crops.

### MATERIALS AND METHODS

Alluvial (entisol), black (vertisol) and red (alfisol) soils were collected from wet lands and eastern block of Tamil Nadu Agricultural University Farm and Kanuvai respectively. The collected soil samples were air dried

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and sifted through a 2 mm sieve. Commercial or experimental formulations of atrazine (atrataf) 2, 4-D Na (Fernoxone) fluchloralin (Basalin), Paraquat (Gramaxone), oxadiazon (Ronstar), oxyfluorfen (Goal), Pendimethalin (Stomp), and Terbutyrn (Terbutrex) were applied at 0.01, 0.1, 1.0, 5.0 and 10.0 ppm so as to cover the usual dosage of 0.5 to 4.0 Kg/ha under field conditions. The soil was mixed immediately to uniformly distribute the herbicide. Twenty viable seeds of cucumber were dibbled in each pot and watered uniformly. Each treatment was replicated three times and bioassays repeated twice. The germination count was taken after emergence and the plants were thinned to 5 plants in order to avoid the competition effect.

The plant height was recorded 15 days after sowing. The plants were pulled out 4 weeks after and the dry matter production was recorded. The collected data were analysed statistically using partial regression analysis (Panse and Sukatme, 1957) and the results are presented.

## RESULTS AND DISCUSSION

The physico-chemical properties of the soil groups tried are given in Table 1.

The germination plant height and dry matter production of cucumber as influenced by herbicide application are presented in Table 2. Increasing concentrations of atrazine, oxadiazon, oxyfluorfen and terbutyrn decreased the

Table 1: Physico-chemical properties of the soils

Sl No.	Particulars	Alluvial	Black	Red
1.	Place of collection	Wetlands TNAU Farm	Eastern Block TNAU Farm	Kanuvai, Coimbatore Dist.
2.	Soil series	Noyyal	Perianaicken- palayam	Somayanur
3.	Soil pH (1 : 2.5 suspension)	7.5	8.4	7.7
4.	Electrical Conductivity (m.mhos/cm)	0.7	1.8	0.6
5.	Available N (Kg/ha)	218	264	148
6.	Available P (Kg/ha)	13.4	16.6	14.5
7.	Available K (Kg/ha)	385	484	218
8.	Organic carbon (%)	0.73	0.98	0.43
9.	Mechanical analysis:-			
	a. Clay	36.4	34.8	21.6
	b. Silt	17.2	16.5	8.4
	c. Fine sand	18.5	20.2	50.8
	d. Coarse sand	24.2	26.9	18.8
10.	Textural Classification	Clay loam	Clay loam	Sandy loam

Table 2: Germination, plant height and dry matter production of cucumber as influenced by herbicide application in different soils.

Herbicides and Treatments (ppm)	Germination percentage (transformed values)			Plant height (cm)			Dry matter production (g/pot)		
	Alluvial	Black	Red	Alluvial	Black	Red	Alluvial	Black	Red
i. Atrazine									
0.0	86.37	81.37	86.37	8.60	13.8	8.40	8.63	7.40	7.73
0.01	68.86	57.00	63.93	6.60	11.2	7.50	7.83	2.40	3.90
0.1	61.21	71.07	51.64	6.43	10.4	7.00	7.40	3.70	3.33
1.0	55.36	52.86	33.93	4.73	7.0	5.0	5.53	1.43	2.53
5.0	42.99	46.71	3.63	3.33	—	—	3.30	—	—
10.0	26.07	38.86	3.63	2.22	—	—	1.87	—	—
ii. 2, 4-D									
0.0	86.37	76.50	81.44	7.83	12.33	8.67	7.70	5.53	7.03
0.01	81.44	59.00	76.51	8.00	9.50	8.00	6.37	4.00	5.53
0.1	71.57	45.00	57.00	6.13	8.33	6.40	5.57	2.73	4.30
1.0	61.92	37.22	56.79	6.73	7.67	7.07	4.37	2.67	3.63
5.0	54.78	33.00	62.64	6.20	2.50	6.57	3.97	0.80	3.57
10.0	48.93	61.21	57.70	5.37	0.83	6.23	3.56	1.26	3.10
iii. Fluchloralin									
0.0	86.37	76.51	81.44	8.17	12.33	8.67	8.13	6.00	7.63
0.01	76.50	78.82	71.57	7.13	10.83	6.97	7.50	5.40	6.67
0.1	71.07	59.71	66.86	6.73	9.50	6.90	6.60	4.67	6.57
1.0	73.79	59.00	81.44	6.37	7.67	7.33	5.73	3.73	6.77
5.0	68.86	52.72	66.64	6.06	5.00	6.77	4.97	2.33	3.50
10.0	61.22	46.92	59.00	5.16	0.83	6.20	3.57	1.97	2.77
iv. Oxadiazon									
0.0	81.44	86.37	78.72	9.40	12.83	9.33	8.83	5.77	9.23
0.01	66.14	47.92	61.21	8.27	11.34	7.03	6.20	5.07	6.33
0.1	59.21	43.08	52.86	7.67	5.60	6.60	5.77	4.47	3.50
1.0	43.08	41.15	34.14	6.40	2.73	5.50	3.57	1.60	2.53
5.0	26.07	39.15	13.50	4.80	—	3.00	3.33	—	0.83
10.0	3.63	35.01	3.63	—	—	—	—	—	—
v. Oxyfluorfen									
0.0	86.37	78.72	86.37	10.22	14.83	8.67	7.83	7.33	6.27
0.01	81.44	52.26	86.37	8.27	12.37	8.17	6.73	6.33	6.33
0.1	78.72	46.21	76.51	7.20	11.57	6.87	5.77	7.13	5.20
1.0	71.57	41.07	66.86	7.03	9.17	6.33	4.97	5.07	5.00
5.0	46.92	37.22	57.00	6.13	9.50	5.00	3.83	4.83	2.43
10.0	27.36	30.00	39.28	2.27	7.50	2.33	1.67	1.47	1.23

	1	2	3	4	5	6	7	8	9	10
vi. Paraquat										
0.0	86.37	13.79	86.37	9.70	14.83	9.20	7.87	8.07	7.17	
0.01	86.37	83.01	86.37	9.90	12.83	9.53	6.90	6.97	7.67	
0.1	86.37	71.57	86.37	10.13	11.67	9.83	7.50	6.00	7.57	
1.0	86.37	81.44	86.37	8.97	9.33	9.27	7.13	6.17	6.50	
5.0	78.72	76.51	71.57	7.40	8.33	8.50	6.90	6.50	6.50	
10.0	86.73	81.43	76.51	8.47	9.77	9.77	7.57	5.73	7.90	
vii. Pendimethalin										
0.0	86.37	73.79	86.37	10.03	15.00	9.33	7.43	6.67	6.50	
0.01	86.37	76.50	86.37	9.43	13.12	8.90	6.13	5.17	6.00	
0.1	81.44	71.58	76.21	9.60	12.50	8.89	6.50	6.00	5.93	
1.0	81.44	61.22	69.57	8.63	10.17	.47	6.67	4.83	6.27	
5.0	71.57	61.72	59.71	10.03	9.67	8.03	7.20	4.07	5.60	
10.0	66.14	63.93	54.99	8.77	6.40	8.20	6.93	3.00	5.87	
viii. Terbutyrin										
0.0	86.37	85.39	86.37	9.33	12.50	10.17	8.03	6.83	5.30	
0.01	81.44	68.86	73.79	8.83	10.83	8.77	7.17	5.33	6.03	
0.1	76.50	66.64	71.08	7.73	7.50	7.57	6.20	5.73	6.37	
1.0	61.72	53.07	59.21	6.47	2.50	7.50	5.50	3.00	5.67	
5.0	53.07	44.92	48.93	5.43	1.17	6.00	4.20	0.83	4.53	
10.0	43.08	33.00	28.28	4.53	-	4.70	3.63	-	2.00	

germination percentage, plant height and dry matter production of cucumber. While the 2, 4 D Na salt, fluchloralin, paraquat and pendimethalin did not affect the above parameters. Atrazine persistence in soil was also studied by Libik and Romanowski (1976) who reported that cucumber seedlings were suitable because they were sensitive enough to detect smaller concentrations of atrazine. The present results are in accordance with the findings of Libik and Romanowski (1976). Fadayomi and Warren (1977), Joques and Harvey (1979) confirm that the herbicides oxyfluorfen and fluchloralin were phytotoxic in their bioassay studies. The effect of paraquat on germination was not seen in our studies and was supported by Appleby and Brenchley

(1968) who observed that germination of legume sp was not reduced even when the seeds were exposed directly to paraquat spray. Da Silva *et al.*, (1976) reported that 2, 4-D was not active at  $1 \times 10^{-4}$  M concentration and True-love *et al.*, (1974) also found no activity for 2, 4-D by cotyledon disc bioassay.

With regard to soil groups the red soil was sensitive than the black and alluvial soils. The probable reason for this might be the lowest clay content and the immediate availability of the herbicide in red soil. Fadayomi and Warren (1977) and Libik and Romanowski (1976) also found similar results in their experiments.

Table-3 : Regression coefficients (R<sup>2</sup>) for the various herbicides under cucumber bioassay

Sl. No.	Herbicides	Germination percentage		Plant height		Dry matter production				
		Alluvial	Black	Alluvial	Black	Alluvial	Black	Red		
1.	Atrozone	0.795**	0.514**	0.722**	0.887**	0.926**	0.932**	0.792**	0.492**	0.683**
2.	2, 4-D Na	0.404*	0.659**	0.138NS	0.232NS	0.755**	0.215NS	0.571**	0.536**	0.350*
3.	Fluchloralin	0.650**	0.546**	0.240NS	0.651**	0.828**	0.260NS	0.768**	0.619**	0.738**
4.	Glyphosate	0.268NS	0.067NS	0.529**	0.552**	0.334*	0.197NS	0.079NS	0.111NS	0.100NS
5.	Oxadiazon	0.881**	0.385NS	0.807**	0.851**	0.715**	0.725**	0.749**	0.853**	0.599**
6.	Oxyfluorfen	0.850**	0.268NS	0.809**	0.757**	0.577**	0.735**	0.763**	0.703**	0.860**
7.	Fendimethalin	0.740**	0.245NS	0.504**	0.187NS	0.629**	0.144NS	0.070NS	0.511**	0.093NS
8.	Terbutryn	0.750**	0.765**	0.845**	0.579**	0.701**	0.697**	0.713**	0.695**	0.853**

\* Significant at 5 per cent level

\*\* Significant at 1 per cent level

NS Not significant

Table 4: LD 50 for germination of cucumber for various herbicides. (ppm)

Sl. No.	Herbicides	Alluvial	Black	Red
1.	Atrazine	2.40	2.80	0.95
2.	2, 4-D Na	3.10	3.00	5.76
3.	Fluchloralin	4.10	4.52	5.80
4.	Oxadiazon	2.47	1.24	0.98
5.	Oxyfluorfen	4.08	3.00	4.10
6.	Paraquat	> 10	> 10	> 10
7.	Pendimethalin	> 10	> 10	> 10
8.	Terbutyrn	4.00	2.95	4.69

The regression coefficients ( $R^2$ ) for the various herbicides are given in Table 3 through which the quadratic equations can be drawn. The LD 50 values i.e. the concentration required to kill 50 per cent of the population were worked out based on the quadratic equations and are presented in Table 4. The LD 50 values for atrazine, oxadiazon and oxyfluorfen were very low indicating that even lower concentrations of these herbicides were sufficient to cause 50 per cent mortality. Red soil has the lowest LD 50 values for many of the herbicides, indicating the acute toxicity even at lower concentrations. The present study will be useful to find out the concentration of a herbicide in the unknown substrate by conducting similar simple bioassay with cucumber plants and computing the parameters like germination, plant height and dry matter production with the available quadratic equations.

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