

**DETERMINATION OF RESIDUE OF HERBICIDES APPLIED IN COTTON THROUGH SORGHUM AND CUCUMBER BIOASSAY**

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**ABSTRACT**

**Objectives:** The study aimed persistence of herbicides through bioassay technique and to determine residual phytotoxicity of different herbicides on succeeding summer crops.

**Methods:** In this study, pot studies and evaluating herbicides residue in soil condition. The post-harvest soil samples from the selected treatments were collected and used for bioassay studies. The collected soil samples of 1-kg soil were filled in clean plastic pots according to the treatments. Another pot culture experiment was conducted with the same soil from the surrounding area of the farm which was never treated with any herbicides.

**Results:** Residues of herbicides in the post-harvest soil were worked out from the bioassay of sorghum and cucumber. In propaquizafop 0.050 kg/ha POE at 45 DAS application showed an average of more herbicide residues and pendimethalin 0.900 kg/ha PE and oxyfluorfen 0.180 kg/ha PE found an average less herbicide residues in the post-harvest soil.

**Conclusion:** All the herbicides were found non-detectable level herbicides residue in the post-harvest soil at germination percentage, plant height, and dry matter production of sorghum and cucumber during both years. Residues by this method for pendimethalin, oxyfluorfen, oxadiargyl, quizalofop-ethyl, imazethapyr, and propaquizafop were non-detectable level (NDL) for all the herbicides.

**Keywords:** Herbicides, Herbicides residue, Sorghum, Cucumber, Bioassay.

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**INTRODUCTION**

Bioassay is a major tool for quantitative and qualitative determination of herbicide residue. In this method, the property of a chemical is measured in terms of some biological responses. Sensitive plant species called as indicators or test species are used for conducting bioassay. Sorghum and cucumber proved suitable bioassay for studies of different herbicide residues in soil under different climates and soil conditions. The major advantages of these techniques are that the procedure followed is generally more economical and easy to perform, and costly equipment is not required. The indicator plant is grown in a field and pot and is compared with that of similar plants grown

in untreated soil. Bioassay methods allow for a rapid assessment of “biologically active” materials throughout a soil core. However, the herbicide could be chemically present, but because of soil binding, might not be biologically active; or, a biological assessment might be the result of two or more chemicals interacting.

Cotton is known as “white gold” and premier commercial crop in India. There are some production problems in cotton cultivation. Weed infestation timing also affects cotton yield. Early weed infestation in cotton crops is one of the major constraints limiting the establishment of the crop and thereafter its production. Competition in the early

**Table 1: Germination, plant height and dry matter of sorghum and cucumber as affected by the residue of herbicides applied to cotton (Pooled 2-year data)**

Treatment	Sorghum			Cucumber		
	Germination %	Plant height (cm)	Dry matter (g)	Germination %	Plant height (cm)	Dry matter (g)
Pendimethalin 0.900 kg/ha pre-emergence	82.00	11.66	0.65	83.25	4.80	0.72
Oxyfluorfen 0.180 kg/ha pre-emergence	83.50	11.94	0.67	80.00	4.88	0.74
Oxadiargyl 0.090 kg/ha pre-emergence	80.50	10.92	0.59	79.75	4.54	0.65
Quizalofop-ethyl 0.040 kg/ha post-emergence at 45 DAS	82.00	11.65	0.63	80.00	4.73	0.69
Imazethapyr 0.075 kg/ha post-emergence at 45 DAS	77.75	10.69	0.52	77.25	4.47	0.57
Propaquizafop 0.050 kg/ha post-emergence at 45 DAS	78.00	10.83	0.56	79.75	4.51	0.62
Glyphosate 0.96 kg/ha post-emergence at 75 DAS (directed spray)	77.25	10.54	0.48	76.75	4.39	0.53
Herbigation of pendimethalin 0.900 kg/ha at 60 DAS (with irrigation water)	79.13	11.12	0.59	80.50	4.57	0.65
Weed free	83.25	12.10	0.69	85.00	4.92	0.76
Unweeded control	78.50	11.13	0.62	79.00	4.62	0.69
S.Em.±	2.72	0.33	0.03	3.84	0.17	0.03
CD (p=0.05)	NS	NS	NS	NS	NS	NS

**Table 2: Germination, plant height and dry matter of sorghum and cucumber as affected by application of different herbicides (Pooled 2-year data)**

	Concentration (g/ha)	Sorghum			Cucumber		
		Germination %	Plant height (cm)	Dry matter (g)	Germination %	Plant height (cm)	Dry matter (g)
Pendimethalin	900	73.50	7.50	0.50	60.00	3.20	0.55
	675	74.75	7.82	0.54	62.50	3.35	0.60
	506	75.00	9.14	0.53	72.00	3.93	0.61
	380	76.25	8.88	0.55	66.00	4.53	0.64
	285	76.50	9.50	0.56	74.50	4.56	0.65
	214	77.75	9.57	0.58	74.75	4.70	0.67
	160	78.75	10.05	0.63	76.25	4.77	0.68
	120	79.75	10.14	0.63	77.75	4.83	0.68
	90	81.25	10.50	0.64	78.25	4.88	0.70
Oxyfluorfen	68	81.75	10.53	0.66	78.50	4.89	0.72
	180	72.50	6.75	0.47	52.50	3.58	0.55
	135	73.50	6.98	0.52	56.50	3.68	0.58
	101	75.00	7.18	0.51	60.00	3.85	0.59
	76	75.75	7.44	0.55	68.75	4.40	0.63
	57	77.00	7.93	0.57	72.50	4.30	0.61
	43	77.50	7.84	0.57	72.75	4.49	0.63
	32	78.00	8.83	0.60	74.25	4.55	0.66
	24	78.50	8.95	0.61	74.25	4.64	0.67
Oxadiargyl	18	79.00	9.53	0.62	74.75	4.65	0.67
	14	79.25	10.10	0.63	75.00	4.69	0.68
	90.0	59.00	5.60	0.38	50.00	3.35	0.43
	67.5	61.25	6.08	0.42	52.75	3.63	0.55
	50.6	64.00	6.80	0.49	57.50	3.40	0.50
	38.0	64.50	7.58	0.45	59.75	3.96	0.56
	28.5	74.75	7.62	0.50	73.00	4.08	0.60
	21.4	74.00	8.04	0.53	69.50	4.34	0.63
	16.0	76.50	8.58	0.56	73.25	4.43	0.65
Quizalofop-ethyl	12.0	77.00	8.53	0.58	74.50	4.54	0.65
	9.0	77.25	9.37	0.60	74.75	4.58	0.67
	6.8	79.00	9.90	0.62	75.50	4.61	0.68
	40.0	75.00	7.50	0.48	71.50	3.40	0.58
	30.0	78.00	8.00	0.54	73.00	3.45	0.61
	22.5	78.50	8.50	0.57	74.50	4.15	0.65
	16.9	80.75	9.49	0.56	76.25	4.28	0.63
	12.7	80.00	9.48	0.59	76.50	4.66	0.68
	9.5	82.00	9.55	0.61	77.00	4.67	0.67
Imazethapyr	7.1	79.75	10.05	0.62	77.35	4.77	0.72
	5.3	80.25	10.35	0.64	78.25	4.78	0.71
	4.0	82.25	10.53	0.65	79.25	4.84	0.72
	3.0	82.75	11.15	0.68	80.25	5.05	0.75
	75.0	72.00	8.00	0.43	67.50	3.08	0.54
	56.3	73.25	8.08	0.48	69.00	3.03	0.58
	42.2	73.50	8.70	0.55	70.00	3.29	0.60
	31.6	75.00	8.73	0.55	71.25	4.32	0.63
	23.7	75.50	8.68	0.55	71.50	4.34	0.63
Propaquiza-fop	17.8	75.75	9.29	0.58	73.75	4.46	0.65
	13.3	77.25	9.58	0.58	74.00	4.47	0.68
	10.0	77.00	9.78	0.61	75.75	4.62	0.66
	7.5	79.00	10.05	0.61	75.75	4.65	0.69
	5.6	79.50	10.15	0.64	77.25	4.76	0.72
	50.0	59.00	9.00	0.55	62.50	3.08	0.44
	37.5	62.50	9.00	0.56	67.00	3.38	0.53
	28.1	72.50	9.32	0.57	68.75	3.95	0.57
	21.1	64.75	9.60	0.60	71.75	4.58	0.64
	15.8	75.75	9.45	0.60	72.75	4.40	0.59
	11.9	76.50	9.78	0.61	74.75	4.76	0.67
	8.9	77.75	9.95	0.62	76.00	4.56	0.65
	6.7	79.00	10.13	0.63	76.50	4.80	0.68
	5.0	79.00	10.27	0.64	77.25	4.84	0.71
	3.8	79.50	10.34	0.65	78.00	4.90	0.72

stages of plant growth is of more concern than weed infestation later in the year. Some weeds, such as broadleaf weeds, germinate and grow quickly. These weeds can establish root systems quickly and use much of the water and nutrients resulting in cotton plants not being able to develop (Chandler, 1983). Cotton losses due to the presence

of weeds may occur in several ways, although damage caused is not always as obvious as losses caused by other pests. Under the present situation of the non-availability of labor for timely weeding and the high costs involved therein, it has become very difficult to maintain cotton crops free from mixed flora of weeds, particularly in the initial

**Table 3: Linear Regression Equation, correlation coefficient (R<sup>2</sup>) of indicator crops (dependent variable Y) with different herbicide applied directly (independent variable X) (Pooled 2-year data)**

Herbicides	Indicator crops	Biometric Parameters	Linear Regression equation	**R <sup>2</sup>
Pendimethalin	Sorghum	Germination %	y=0.922x+72.44	0.950
		Plant height (cm)	y=0.330x+7.540	0.870
		Dry matter (g)	y=0.017x+0.481	0.916
	Cucumber	Germination %	y=2.039x+60.83	0.801
		Plant height (cm)	y=0.189x+3.321	0.804
		Dry matter (g)	y=0.016x+0.558	0.936
Oxyfluorfen	Sorghum	Germination %	y=0.751x+72.46	0.914
		Plant height (cm)	y=0.369x+6.119	0.927
		Dry matter (g)	y=0.016x+0.469	0.903
	Cucumber	Germination %	y=2.534x+54.18	0.798
		Plant height (cm)	y=0.129x+3.567	0.842
		Dry matter (g)	y=0.013x+0.550	0.922
Oxadiargyl	Sorghum	Germination %	y=2.376x+57.64	0.873
		Plant height (cm)	y=0.446x+5.347	0.891
		Dry matter (g)	y=0.025x+0.3	0.903
	Cucumber	Germination %	y=3.063x+49.2	0.810
		Plant height (cm)	y=0.153x+3.243	0.856
		Dry matter (g)	y=0.024x+0.452	0.833
Quizalofop-ethyl	Sorghum	Germination %	y=0.65x+76.35	0.616
		Plant height (cm)	y=0.372x+7.407	0.931
		Dry matter (g)	y=0.095x+0.487	0.917
	Cucumber	Germination %	y=0.878x+71.55	0.919
		Plant height (cm)	y=0.177x+3.43	0.839
		Dry matter (g)	y=0.017x+0.572	0.885
Imazethapyr	Sorghum	Germination %	y=0.801x+71.36	0.962
		Plant height (cm)	y=0.252x+7.710	0.913
		Dry matter (g)	y=0.019x+0.446	0.835
	Cucumber	Germination %	y=1.056x+66.76	0.931
		Plant height (cm)	y=0.204x+2.975	0.808
		Dry matter (g)	y=0.016x+0.541	0.892
Propaquizafop	Sorghum	Germination %	y=2.255x+60.21	0.785
		Plant height (cm)	y=0.159x+8.803	0.961
		Dry matter (g)	y=0.064x+0.541	0.937
	Cucumber	Germination %	y=1.604x+63.69	0.901
		Plant height (cm)	y=0.189x+3.281	0.750
		Dry matter (g)	y=0.026x+0.467	0.829

stage of growth. Hence, herbicidal control of the weeds could assume greater significance. Day-by-day weed control through herbicides is increasing and popularizing among farmers. A number of herbicides are recommended for field crops in the intensive cropping system may lead to residue accumulation in soil. This causes considerable health hazards and environmental pollution. Therefore, persistence studies are essential to determine the duration of herbicidal efficacy and its effect on follow-up crops. This experiment was conducted to determine the residues of different herbicides in soil applied in cotton.

#### METHODS

A field experiment was carried out to determine of residue of herbicides applied in cotton through sorghum and cucumber bioassay at the Department of Agronomy, College of Agriculture, Junagadh Agricultural University, Junagadh (Gujarat) during *Kharif* 2013–2014 and 2014–2015. The trial was arranged in a randomized complete block design and replicated thrice. The seedbed was prepared by cultivating the field two times with tractor-mounted cultivar each followed by planking. The crop was sown on medium black calcareous soil. Sowing was done with the help of a single row hand drill. Experimental ten treatments comprised of cotton crop viz. pendimethalin 0.900 kg/ha PE (T<sub>1</sub>), oxyfluorfen 0.180 kg/ha PE (T<sub>2</sub>), oxadiargyl 0.090 kg/ha PE (T<sub>3</sub>), quizalofop-ethyl 0.040 kg/ha POE at 45 DAS (T<sub>4</sub>), imazethapyr 0.075 kg/ha POE at 45 DAS (T<sub>5</sub>), propaquizafop 0.050 kg/ha POE at 45 DAS (T<sub>6</sub>), glyphosate 0.96 kg/ha POE at 75 DAS (directed spray) (T<sub>7</sub>), herbigation of pendimethalin 0.900 kg/ha at 60 DAS (with irrigation water) (T<sub>8</sub>), Weed free (T<sub>9</sub>) and unweeded check (T<sub>10</sub>). Overall five irrigations were applied and weeds were controlled as per treatments.

All other agronomic practices were kept normal and uniform for all the treatments.

Bioassay is a major tool for quantitative and qualitative determination of herbicide residues. In this method, the property of a chemical is measured in terms of some biological responses. The response is measured through two different types viz., (i) plant part response and (ii) total plant response (Lavy and Santelmann, 1986). The second procedure was adopted in this experiment in which the entire plant may exhibit chlorosis or a suppression or inhibition of growth. This response may be demonstrated by harvesting the sensitive plants (sorghum and cucumber) in pot studies and evaluating growth parameters and herbicide residues in soil conditions. The post-harvest soil samples from the selected treatments were collected and used for bioassay studies. The collected soil samples (15 cm depth) were air-dried, powdered, and passed through a 3-mm sieve. One kg of soil was filled in clean plastic pots according to the treatments. The experiment was replicated thrice. Indicator crops sorghum and cucumber were raised to study the residual effect of herbicides. Ten viable seeds were sown and germination count was taken 7 days after sowing. Then the plant is thinned to 3 plants uniformly in all pots to avoid the competition effect. The viable seeds were sown and germination count was taken 10 days after sowing. The plant height and dry matter production (DMP) were taken after 30 days.

Another pot culture experiment was conducted with the same soil from the surrounding area of the farm which was never treated with any herbicides. Air-dried and processed soil was filled in each plastic pot. The herbicides such as pendimethalin, oxyfluorfen, oxadiargyl,

Table 4: Different herbicide residue in soil by sorghum and cucumber growth bioassay (Pooled 2-year data)

Treatment	Indicator crops	Parameters of indicator plants	Average herbicide residue remaining (mg kg <sup>-1</sup> ) BDL
Pendimethalin 0.900 kg/ha PE	Sorghum	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
	Cucumber	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
Oxyfluorfen 0.180 kg/ha PE	Sorghum	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
	Cucumber	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
Oxadiargyl 0.090 kg/ha PE	Sorghum	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
	Cucumber	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
Quizalofop-ethyl 0.040 kg/ha POE at 45 DAS	Sorghum	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
	Cucumber	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
Imazethapyr 0.075 kg/ha POE at 45 DAS	Sorghum	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
	Cucumber	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
Propaquizafop 0.050 kg/ha POE at 45 DAS	Sorghum	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND
	Cucumber	Germination %	ND
		Plant height (cm)	ND
		Dry matter (g)	ND

Permissible limit: (0.01 mg/kg), Source: Central Insecticidal Board and Registration Committee

ND: (Non-detectable)

quizalofop-ethyl, imazethapyr, and propaquizafop were applied at different concentrations and soil was thoroughly mixed. A standard curve was drawn for different concentrations of different herbicides versus germination percentage, plant height, and DMP, and the linear regression equation and regression coefficients for this curve were calculated. In the field, after harvesting of cotton sown test crops, such as sorghum and cucumber to record the observation for germination percentage, plant height, and DMP. The data were fitted into the linear regression equation for the determination of herbicide residues under field conditions (Shubhendu, 1999 and Rupak *et al.*, 2009).

## RESULTS AND DISCUSSION

The germination %, plant height (cm), and DMP (g) of the sorghum and cucumber plant as affected by herbicide residues are presented in Table 1. Sorghum and cucumber showed good response and were sensitive to herbicide residues. The data pertaining to the bioassay of sorghum and cucumber under varying concentrations of herbicides used are presented in Table 2. The increasing concentrations of herbicides decreased the germination %, plant height (cm), and DMP (g) of the sorghum and cucumber significantly. The regression coefficients ( $R^2$ ) and linear regression equations were worked out for each herbicide (Table 3) during in pooled results. Significant regression coefficients were obtained for all parameters for all the herbicides.

From the linear regression equations, the residues of herbicides in the post-harvest soil were worked out from the bioassay of sorghum

and cucumber, and the data are presented in (Table 4). In 2-year data, propaquizafop 0.050 kg/ha POE at 45 DAS application showed an average of more herbicide residues, and pendimethalin 0.900 kg/ha PE and oxyfluorfen 0.180 kg/ha PE was found to average less herbicides residues in the post-harvest soil, based on the biometric parameters of indicator crops during both the years. While all the herbicides were found non-detectable level herbicides residue in the post-harvest soil at germination percentage, plant height, and DMP of sorghum and cucumber during both years. The residue level in post-harvest soil in cotton is low for herbicide application or herbicide might have dissipated leaving less residue in surface soil.

## CONCLUSION

From the above study, it can be concluded the germination of sorghum and cucumber showed consistent residue levels and this parameter can be best suited for residue estimation of herbicides when applied to cotton. The residue level in post-harvest soil of cotton is low and this may be due to the very 1<sup>st</sup> and 2<sup>nd</sup> year of herbicide application or herbicides might have leached down to the subsurface soil with rain water and thereby leaving less residue in the surface soil. There was no residual toxicity of above-mentioned pre- and post-emergence herbicides through field bioassay after harvesting of cotton and it was safe to grow succeeding crops. These findings are in conformity with those reported by Arora and Gopal (2004), Sondhia (2008), Sushil *et al.* (2008), Mukhopadhyay *et al.* (2012), Singh *et al.* (2013), Mandal *et al.* (2014) and Singh and Vaishya (1993).

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