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STANDARDIZATION OF BIOASSAY TECHNIQUE FOR DETERMINING BUTACHLOR RESIDUES IN KOLE SOILS OF KERALA

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Abstract: Different plant species were grown in pots containing soil fortified with known quantities of butachlor and based on the response to the herbicide amaranth was selected as the indicator plant for the herbicide. Among the several growth parameters tested for assessing the plant response to soil residues of butachlor, CSGR (compound shoot growth rate) proved to be the best for quantitative determination of butachlor residues in kole soils of Kerala.

Key words: Bioassay, butachlor residues, kole soils, compound shoot growth rate.

INTRODUCTION

The kole lands cover an area of more than 13,000 ha, spread over Thrissur and Malappuram districts of Kerala. The productivity of rice crop in the kole area is perhaps the highest in the state. The soils have varying soil properties. The texture ranged widely from sandy loam to clay. Other characteristics were pH 2.3 to 8.19; EC 0.09 to 9.25 dS m^{-1}; organic carbon 0.12 to 9.93 %; total nitrogen 0.04 to 0.98 %; total P 0.012 to 0.144; total K 0.04 to 0.432 %; sesquioxide 6.4 to 35.2 % and CEC 4.4 to 49.3 cmol(+) kg^{-1} (Ambili, 1995).

In majority of the rice growing areas of kole lands, direct sowing in puddled field is the practice followed by farmers. Transplanting has almost been replaced by direct sowing due to the scarcity and high cost of labour. Weed control is a serious problem in direct sown crop and butachlor @ 1.25 kg ha" is recommended for pre-emergence application (KAU, 1997). From the results of various studies conducted in different parts of the country it was found that in paddy soil butachlor persisted up to 66 days and the half-life was about 17.8 days (Sankaran *et al.*, 1993). At the time of harvest, no detectable amount was found in the soil. Studies on the determination of butachlor residues in paddy soils have not been carried out in Kerala so far. Since bioassay is the simplest technique for the detection of butachlor residues, an experiment was planned to standardize the bioassay system for the detection of butachlor residues in the rice fields.

MATERIALS AND METHODS

Representative soil samples from kole lands of Thrissur District where herbicide had never been used for weed control were collected immediately after the harvest of the summer paddy. Soil samples were air dried, processed and their major physiochemical characteristics were estimated. Pooled samples were taken uniformly (1.5 kg) in clean mud pots of size 20 cm x 18 cm The holes were sealed to prevent drainage.

Bioassay was standardised in two steps, viz., (a) preliminary study and (b) detailed study. The preliminary study consisted of three levels of butachlor (0 ppm, 1 ppm and 2 ppm) and seven indicator plants (cucumber, amaranth, bhindi, cowpea, greengram, blackgram and ragi).

The experiment was conducted in completely randomised design with five replications. Based on the response to the herbicide, most suitable indicator plant was selected.

The detailed study was conducted with wider range of concentrations of the herbicide in soil medium. The treatments consisted of seven levels of herbicide viz., 0, 0.1, 0.21, 0.44,0.92, 1.91 and 4.00 ppm of butachlor in completely randomized block design with three replications. Soil was fortified with butachlor as per the treatments and tO seeds of selected crop species were dibbled in each pot at uniform depth of 2 cm. The pots were watered regularly to near field capacity, without draining.

Table 1. Effect of soil applied butachlor on the shoot length of test plants at different stages, cm

Crop	14 DAS			21 DAS			28 DAS			35 DAS		
	Rate of application kg ha^{-1}			Rate of application kg ha^{-1}			Rale of application kg ha^{-1}			Rate of application kg ha^{-1}		
Cucumber	9.96	7.93	8.40	13.00	9.97	10.67	15.47	11.65	12.77	19.73	13.87	13.57
CD (0.05)		MS			NS			NS			NS	
Amaranth	2 13	1 87	1.85	2.53	2.30	2.00	2.80	2,47	2.19	3,67	2.73	2.40
CD (0.05)		NS			NS			NS			0.82	
Bhindi	14.93	15.47	15.27	15.97	16,87	16.93	18.37	19.20	19.42	21.50	20.58	20.41
CD (0.05)		NS			NS			NS			NS	
Cowpea	19.60	18.77	19.00	21.03	20.63	20.53	23.91	22.70	22.93	26.47	24.67	24.23
CD (0.05)		NS			MS			NS			NS	
Greengram	15.20	16.13	19.83	16.07	17.43	19.17	17.50	19.17	20.93	18.40	20.17	22.02
CD (0.05)		1.41			1.40			1 45			1.95	
Blackgram	12 S3	13.61	12.60	14.67	16.60	15.33	16.50	18.80	17.17	17.90	21.73	18.63
CD (0.05)		NS			NS			NS			NS	
Ragi	1 43	1 37	1.07	1.96	1.67	1.43	2.90	2.80	2.20	3.87	3 86	3.40
CD (0.05}		NS			NS				NS		NS	

Germination count was taken one week after sowing and then the plants were thinned to three plants per pot, to avoid competition. Plant height was recorded from 14 DAS (days after sowing) to 35 DAS, at weekly intervals. The plants were cut close to the soil at 35 DAS, dried in a hot air oven at 80°C and the dry weight was recorded. The data were analysed statistically.

Table 1. Effect of butachlor applied (0, 1 and 2 ppm) to soil on germination, dry weight and compound shoot growth rate in different test plants

Test plant	Germination (%)			Dry weight of plants (g)			Compound shoot growth rate (%)		
	Contr Ol	1 ppm	2 ppm	Control	Ippm	2 ppm	Control	1 ppm	2 ppm
Cucumber	96.67	86.67 (10.34)	80.00 (17.24)	0.218	0.126 (42.20)	0.126 (42.20)	25	20 (20)	18 (28)
Amaranth	96.67	96.67 (0)	86.67 (10.34)	0.054	0.022 (59.26)	0.016 (70.37)	19	13 (32)	9 (53)
Bhindi	96.33	90.00 (7.37)	96.67 (-0.35)	0.362	0.238 (34,25)	0.260 (28.18)	13	10 (23)	1[(15)
Cowpea	100.0	100.00 (0)	96.67 (3.33)	0.652	0.&02 (7.67)	0526 (19.32)	10	8 (20)	8 (20)
Greengram	100.0	96.67 (3.33)	100.00 (0)	0.132	0.230 (-74.24)	0.150 (-3.63)	6	7 (-17)	7 (-17)
Blackgram	100.0	100.00 (0)	100.00 (0)	0.172	0.252 (-46.51)	0.176 (-2.33)	10	15 (-50)	12 (-20)
Ragi	96.67	8333 (13,80)	66.67 (31.03)	0,034	0.066 (-94.12)	0.032 (58,82)	35	38 (-8)	33 (6)

Percentage reduction in growth rate from control are given in parentheses

RESULTS AND DISCUSSION

a) Preliminary study

The physicochemical analysis of the soil samples under study indicated that the soil pH ranged from 4.5 to 5.0; EC 0.5 to 2.5 dS m^{-1}; organic carbon 1,5 to 2.0 %; available P 14 to 18 ppm; available K 340 to 360 ppm; CEC 21 to 25 cmol(+) kg^{-1} and the texture was sandy clay. The statistical analysis of the data obtained from the preliminary study showed

Table 3. Response of amaranth to soil residues of butachlor

(Conc. of butachlor in soil) (ppm)	Germination count		Dry wt. of plants (mg)	Shoot length (cm) at				Compound shoot growth rate (%)
	O	T		14 DAS	21 DAS	28 DAS	35 DAS	
0.00	9.67	1.36 (0)	3.00 (0)	2.33 (0)	3.17 (0)	3.50 (0)	4.33 (0)	21.62 (0)
0.10	9.67	1.36 (0)	3,00 (0)	2.17 (6.87)	2.92 (7,88)	3.23 (7.71)	4.00 (7.62)	21.33 (1.34)
0.21	9.33	1.30 (4.4)	2.67 (11.00)	1,92 (17.60)	2,50 (21.14)	2.80 (20.00)	3.33 (23.09)	19.40 (10.27)
0.44	9.00	1.25 (8.09)	2.33) (22,33)	1.83 (21.46)	2.46 (2240)	2.67 (23.71)	3.00 (30,72)	16.95 (21.60)
0.92	7.67	1.07 (21.32)	2.17 (27.67)	1.75 (24.89)	2.33 (26.50)	2.58 (26.29)	2.78 (35.80)	16.15 (25.30}
1.91	7.33	1.03 (24.26)	1.83 (39.00)	1.67 (28.33)	1.92 (39.43)	2.21 (36.86)	2.42 (44.11)	13.24 (38.76)
4.00	7.3.1	1.03 (24.26)	1.00 (66.67)	1.50 (35,62)	1.75 (44.79)	1.87 (46.57)	1.92 (55.66)	8.39 (61.19)
CD (0.05)		0.136	0.6895	0.3955	0.308	0.418	0.4954	
Correlation coefficient (r) with treatments		-0.920*	-0.996*	-0.923*	-0.980*	-0,967*	-0.966*	-0.985*

O = Original values T = $Sin^{-1}\sqrt{P}$ transformed values; Percentage reduction from the control are given in parentheses

that butachlor at 1 ppm decreased the shoot growth of cucumber, amaranth, bhindi and cowpea (Table 1), An increase in the level of butachlor from 1 ppm to 2 ppm resulted in further reduction in the shoot growth of cucumber and amaranth. Germination and dry weight of plants did not differ signify-cantly between treatments (Table 2).

Estimates on compound shoot growth rate were obtained by getting an exponential equation of the form $Y = AB^t$ where Y = shoot length in cm; t = time in number of weeks starting from the second week after sowing; A == the intercept; B= compound shoot growth rate. B was estimated as (B′-1) x 100 where B′ is the least square estimate of B from the log linear model corresponding to the exponential model. Among the different crop species tested, cucumber and amaranth showed greater response to the herbicide in terms of shoot growth rate over a period of 14 to 35 DAS. For the concentrations tried viz; 1 ppm to 2 ppm, the percentage reduction in shoot growth rate from control was 32 to 53 % and 20 to 28 % for amaranth and cucumber respectively. Thus amaranth is a better indicator plant for the determination of butachlor residues.

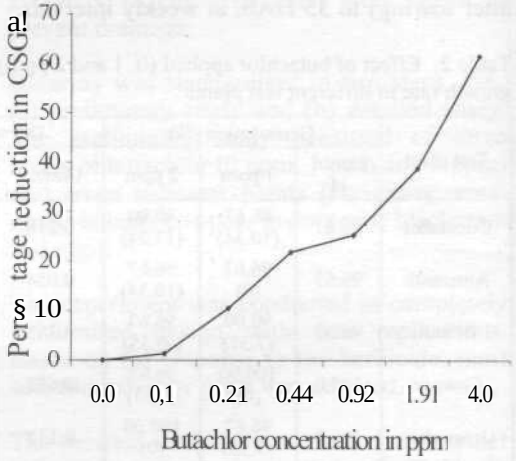

Fig 1, Relationship between percentage reduction in CSGR and butachlor application

b) Detailed study

The bioassay system was further standardised by growing the indicator plant in pots con-

The bioassay system was further standardised by growing the indicator plant in pots containing soil fortified with seven geometrically increasing concentrations of butachlor viz., 0, 0.1, 0.2, 0.44, 0.92, 1.91 and 4.00 ppm. It was noticed that increasing concentrations of the herbicide in the soil progressively reduced the height of amaranth seedlings. This effect was significant for the highest two concentrations (1.91 and 4.00 ppm) from the 14th day (Table 3), Application of the herbicide at 0.1 ppm did not affect the shoot length at any growth stage. The response of amaranth to concentrations higher than 0.1 ppm was maximum at 35 DAS, The germination and dry weight of plants were reduced significantly by the application of butachlor at concentrations above 0,44 ppm. As the results of the preliminary study indicated that comparison of the percentage reduction in CSGR values would be a better estimate of the herbicide residues present in the soil, CSGR from 14 DAS to 35 DAS was computed in the detailed study also. There was significant negative correlation between concentration of the herbicide and germination, length and dry weight of shoots and CSGR of plants (Table 3).

Growth response to the herbicide in terms of percentage reduction in growth over control was estimated for all the parameters and presented in Table 3. The response range was narrow in the case of shoot length at 14 DAS, 21 DAS and 28 DAS and also for germination. Wider range of response was obtained for shoot length at 35 DAS (7.62 to 55.66 %) dry weight of plants (11.00 to 66.67 %) and CSGR (1.34 to 61.19 %). The extent of variation in response was largest in the case of CSGR followed by dry weight of plants. Hence, in the amaranth bioassay for butachlor, CSGR was found to be more sensitive than other parameters such as germination percentage, shoot length at a particular stage or dry weight of plants. From the relationship between percentage reduction in CSGR and levels of butachlor (Fig. 1) the residual amount of butachlor in kole soils can be estimated.

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