EFFECT OF BUTACHLOR ON THE PROTEIN CONTENT OF C-168 AND IR-36 RICE CULTIVARS AT DIFFERENT GROWTH STAGES

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ABSTRACT

Rice seedlings of C-168 cultivar were markedly inhibited by butachlor up to shoot emergence stage while those of IR-36 showed greater inhibition up to the second leaf stage. Treated rice seedlings up to shoot emergence stage have comparatively less weights than the untreated control in both cultivars. Protein synthesis was severely affected by butachlor treatment at pre-germination up to shoot emergence, the degree of inhibition being greater in IR-36 than in C-168. There was no protein synthesis inhibition when seedlings of both cultivars were treated later during the first and second leaf stages.

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KEY WORDS: Rice. Cultivar C-168 and IR-36. Butachlor. Protein content. Different stages of rice plants. Root and shoot elongation.

INTRODUCTION

Butachlor (N-butoxymethyl-2-chloro-2'6' diethylacetanilide belongs to amide group of herbicides. It is a selective, pre-emergence herbicide principally used for the control of most annual seedling-grasses and certain broadleaf weeds associated with rice.

Some amides have been reported to inhibit £-amylase induction by gibberellic acid in barley endosperm (Devlin and Cunningham, 1970). Pillai *et al.* (1979), on the other hand, reported that metolachlor inhibited protein synthesis possibly brought about by inhibition of leucine uptake rather than inhibition of protein synthesis *per se.*

This study was conducted to determine the effect of butachlor on

the protein content of C-168 and IR-36 rice cultivars at different growth stages.

MATERIALS AND METHODS

Treatments. — Rice at different growth stages (pre-germination, radicle emergence, shoot emergence, first leaf stage and second leaf stage) were treated with 10 ml of 50 ppm butachlor for 24 hr in Petri dishes. There were 3 replications per treatment and 15 rice seeds or seedlings in each Petri dish.

After 24 hr, rice seeds (in case of pre-germination) and seedlings were rinsed with distilled water and transferred to another set of Petri dishes containing 10 ml of distilled water. The plants were allowed to grow for one week after which root and shoot lengths were measured. Percent inhibition of both tissues was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Length of control root/shoot - length of treated root/shoot}}{\text{Length of control root/shoot}} \times 100$$

The plant tissues were harvested, weighed, stored at 10°C and utilized for protein analysis.

Extraction of Protein. — One-half gram of plant tissue sample was ground in a mortar and pestle with 25 ml of pre-chilled 0.1 M potassium phosphate buffer (pH 7.8) and squeezed in a cheese cloth. The residue was discarded and the extract was centrifuged at 1500x g for 30 min at 10°C. The crude extract was discarded and the supernatant was utilized for protein determination using the Lowry Method (Lowry *et al.*, 1951).

Estimation of Protein Content with the Folin-Ciocalteu Phenol Reagent. — The protein content of each sample was determined using the Folin-Ciocalteu phenol reagent. The reagents used were:

Reagent A - 2% Na_2CO_3 in 0.1 N NaOH
Reagent B - 0.5% $CuSO_4$-$5H_2O$ in 1% solution tartrate
Reagent C- alkaline copper solution. Mix 50 ml of reagent A with 1 ml of B
Reagent D- Folin-Ciocalteu's phenol reagent 2N, diluted with water to one-half resulting in IN solution

One-half ml of the sample was mixed with 5 ml of reagent C in a test tube. The mixture was allowed to stand for 10 min at room temperature. One half ml of reagent D was added immediately and thoroughly mixed. After 30 min, the samples were read in a spectrophotometer at 500 nm. The protein content was calculated by comparing it with the standard curve, using bovine serum albumin prepared for the working concentrations.

RESULTS AND DISCUSSION

Effeect of Butachlor on Root and Shoot Elongation.

The results showed that IR-36 cultivar is more susceptible to butachlor than C-168 (Table 1). The shoots of C-168 rice cultivar were markedly inhibited up to shoot emergence stage by butachlor while roots were inhibited only up to emergence of the radicle. In the case of IR-36 cultivar, the shoot tissues were markedly suppressed up to the first leaf stage.

The greater effect of butachlor was apparent during the earlier stages of rice growth when the plant rely solely on the food reserve. Seedlings treated at later stages of growth when leaves were already photosynthesizing can overcome partially or totally butachlor phytotoxocity although this depends upon the cultivar. For example, in IR-36 rice cultivar, which is more susceptible, there was still considerable inhibition at later stages. On the other hand, butachlor did not seem to affect elongation of C-168 rice cultivar after the first leaf has emerged.

Table 2 shows the fresh weights of C-168 and IR-36 seedlings treated with butachlor. The results correlate with the foregoing observations. The weights of C-168 cultivar were markedly reduced when treated with butachlor up to shoot emergence stage, while the weights of the untreated and treated plants

Table 1. Root and shoot lengths of IR-36 and C-168 rice seedlings at different developmental stages after 24 hr of butachlor treatment.

Stage of Growth	C-168 CULTIVAR				IR-36 CULTIVAR			
	Length[2] (mm)		% Inhibition		Length[2] (mm)		% Inhibition	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Pre-Germination								
Control	60.33 ••	45.91 ••	—	—	78.92 ••	53.45 ••	—	—
Treated	11.51	8.30	80.92	81.92	6.92	7.09	91.23	86.74
Radicle Emergence								
Control	73.61 ••	54.63 ••	—	—	74.57 ••	55.09 ••	—	—
Treated	37.17	17.45	57.65	68.05	25.54	8.85	65.58	83.94
Shoot Emergence[1]								
Control	48.80ns	49.86••	—	—	77.25 ••	57.26 ••	—	—
Treated	52.42	6.71	7.41	86.54	39.27	5.42	49.17	90.53
First Leaf Stage[1]								
Control	30.66ns	34.84ns	—	—	56.96 ••	48.37 ••	—	—
Treated	31.30	29.73	-2.08	-7.06	42.48	16.06	25.42	66.80
Second Leaf Stage[1]								
Control	2.83ns	40.93ns	—	—	27.86ns	35.00 ••	—	—
Treated	6.03	29.73	-113.7	27.36	26.60	14.26	4.52	59.26

[1] Increments in length were used for computation of data.

[2] Average of 3 replications.

•• Highly significant.

ns Not significant

Table 2. Effect of butachlor on fresh weights of rice seedlings at different growth stages.

STAGE OF GROWTH	FRESH WEIGHT (g)[1]	
	C-168 Cultivar	IR-36 Cultivar
Pre-Germination		
Control	1.136 **	1.154 **
Treated	0.686	0.576
Radicle Emergence		
Control	1.390 **	1.160 **
Treated	0.910	0.712
Shoot Emergence		
Control	1.361 **	1.335 **
Treated	0.965	0.820
First Leaf Stage		
Control	1.345ns	1.542 **
Treated	1.431	1.216
Second Leaf Stage		
Control	1.407ns	1.383 **
Treated	1.332	1.505

[1] Average of 3 replications; data taken 24 hr after treatment.

** Highly significant

ns Not significant

were not significantly different from each other during the first and second leaf stages. In IR-36 cultivar, the treated plants had a highly significant reduction in fresh weights compared with the untreated, except that during the second leaf stage, treated plants were heavier than the untreated control. These results indicate that cell division leading to tissue elongation was affected by butachlor treatment, and because of inhibition of elongation, there was a corresponding reduction in the weights of the treated rice seedlings.

Morphological observations also showed that rice plants affected by butachlor treatment exhibited stunted growth of root and shoot tissues and that the first primary leaf was not able to break through the coleoptile. These findings were similar to that of Mercado, Talatala and Toquero (1975) who found that rice plants treated with butachlor exhibited marked reduction of shoot and root growth. However, recovery from herbicide phytotoxicity was also apparent as the seedlings grew older.

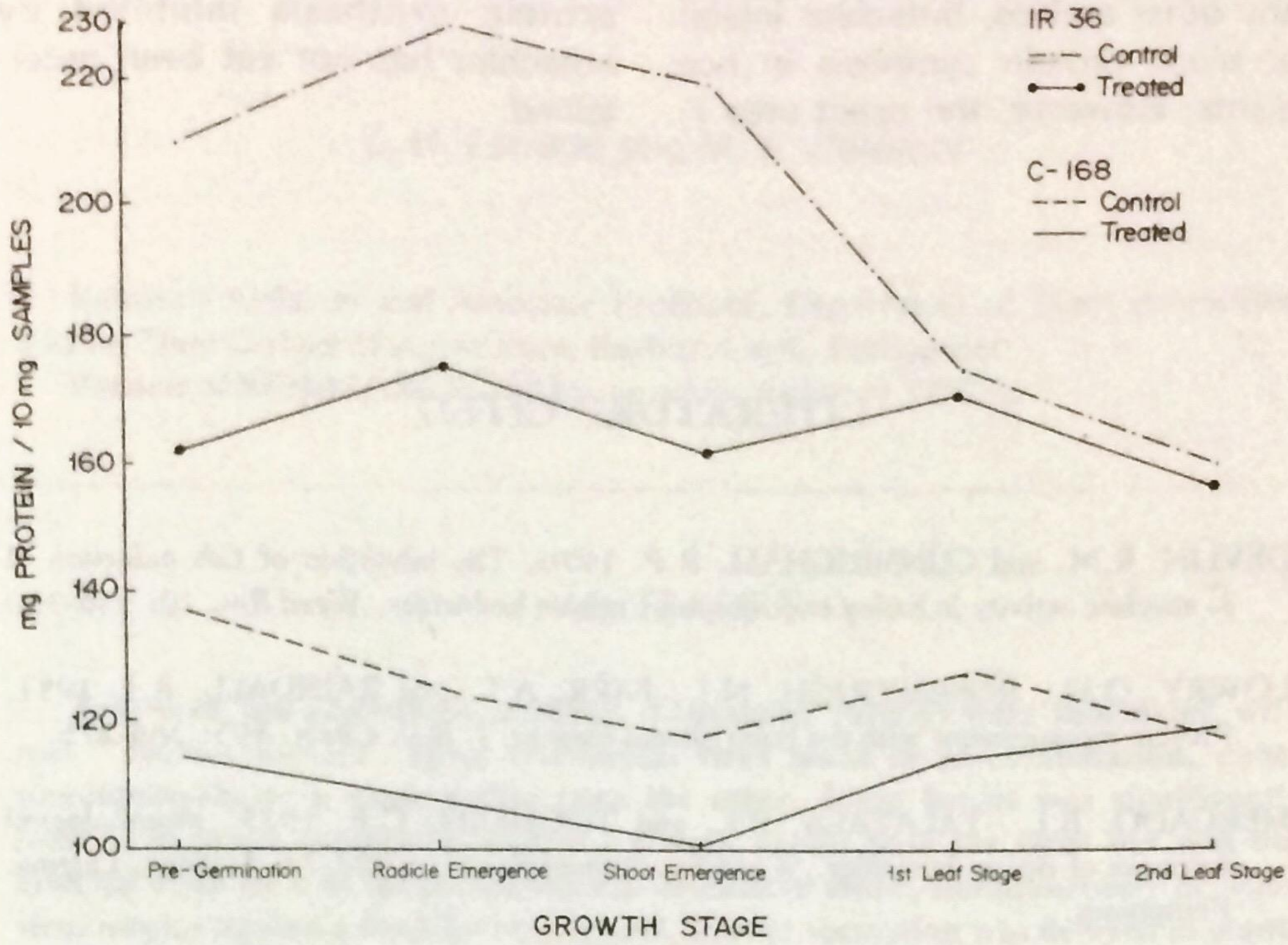

Fig. 1. Protein content of rice at different growth stages as affected by 50 ppm butachlor.

Effect of Butachlor on the Protein Content of Seedlings.

Treatment with butachlor at different growth stages showed that the amount of protein produced by the plant was considerably affected by the herbicide from pre-germination to shoot emergence stage (Fig. 1). Regardless of cultivar used, protein synthesis was not affected by the treatment at later stages of development, that is, from the first to second leaf stage as shown by the non-significant difference on the amount of protein produced between the treated and untreated plants. Being more susceptible to the herbicide, IR-36 cultivar gave higher reduction in protein content due to butachlor treatment. It was very apparent that from pre-germination up to shoot emergence stage there was a greater inhibition of protein synthesis in IR-36 compared to C-168 cultivar at the same stages. Both cultivars responded similarly to butachlor treatment during the first and second leaf stages wherein protein synthesis was either very slightly or not at all affected.

The above results can be correlated with the data presented in Table 1 wherein elongation process was also inhibited during the earlier developmental stages of rice plants. Since cell elongation can be inhibited by inhibiting cell division and that such growth disturbance is very much related to inhibition of protein synthesis, it is assumed that just like

any other amides, butachlor inhibit or block protein synthesis in rice plants. However, the exact step in protein synthesis inhibited by butachlor has not yet been ascertained.

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