Indian J. Weed Sci. **42** (3 & 4) : 223-225 (2010) *Short Communication*

Effect of Rice Herbicides on β-glucosidase, Protease and Alkaline Phosphatase Activity in Soil

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In the present investigation, it was observed that the herbicides tested caused an initial decrease in glucosidase, protease and alkaline phosphatase enzyme activities when applied at recommended rates. But this decrease was transient and short-lived and enzyme activities recovered to the level of control at the end of incubation period of 30 days indicating the resilience of the soil to the perturbation caused by herbicide application at recommended doses.

Pre-emergence or post-emergence application of herbicides results in a large proportion of the herbicides reaching the soil and accumulating in the top 0-15 cm. Herbicides being biologically active compounds may adversely affect soil microorganisms and their activity that greatly contribute to the health and productivity of soils. Hence, there is a need to study the effects of herbicides on soil microbial communities thereby addressing the apprehensions about the environmental impacts of herbicide use.

With this background, the present investigation was carried out with the objectives to understand and predict the effect of herbicides viz., 2,4-D-2ethylhexyl ester (2,4-DEE), butachlor, pretilachlor and pyrazosulfuron ethyl on enzymatic activities in rice soils, which could lead to their judicious use thereby reducing their negative effects, if any on the environment. The enzymes β-glucosidase, protease and alkaline phosphatase involved in the cycling of carbon, nitrogen and phosphorus were examined for their response towards herbicides applied at different concentrations.

A laboratory incubation experiment was conducted using field soil obtained from wetlands of TNAU, Coimbatore, by devising microcosms to study the effect of different concentrations of herbicide formulations on enzyme activities. The soil was air-dried and passed through 2 mm sieve. The soil portions equivalent to 250 g oven dry weight were placed in 500 ml beakers, adjusted to the required level of moisture in flooded condition and pre-incubated at 30±1°C for three days for conditioning. Appropriate quantities of the herbicide formulations were added to the soil to maintain concentrations of herbicides at control, FR (Field rate), 2 FR (2 x Field rate), 5 FR (5 x field rate), 10 FR (10 x field rate) and 100 FR. Soil without herbicide application was also maintained as control. The field rates of application for different herbicides were 0.75 kg/ha for 2,4-DEE, 1.0 kg/ha for butachlor, 0.30 kg/ha for pretilachlor and 25 g/ha for pyrazosulfuron-ethyl. Water was adjusted to the same level in all the treatments including control. A 3 cm depth of overlying water was maintained in all the treatments. The treated soils were then covered with plastic sheets having small holes and incubated at 30±1°C in the dark for 30 days. Soil samples were drawn at 0, 7, 15 and 30 days after application of herbicides and analysed for the effect of herbicides on enzyme activities in soil.

β-glucosidase assay was based on the colorimetric determination of p-nitrophenol released by β-glucosidase when soil was incubated with buffered (pH 6.0) p-nitrophenyl β-D glucopyranoside (Eivazi and Tabatabai, 1988). Protease activity was assayed by determining the tyrosine released when soil sample was incubated with sodium caseinate as the substrate (Ladd and Butler, 1972). Soil alkaline phosphatase activity was assayed by the method of Tabatabai and Bremner (1969).

The enzyme activities were significantly affected by the type of herbicides, concentration of herbicides and days after application of herbicide (Table 1). Among the herbicides, it was observed that butachlor was more inhibitory to soil enzyme activities (5.03 to 19.11% reduction over control) when compared to 2, 4-DEE, pretilachlor and pyrazosulfuron ethyl. The lowest activity of glucosidase (66.56 µg p-nitrophenol/g soil/h), protease (27.13 mg tyrosine/g soil/h) and alkaline phosphatase (99.58 µg p-nitrophenol/g soil/h) was recorded after butachlor application. Herbicides applied at 100 FR concentrations were observed to be more inhibiting (6.74 to 31.27 % reduction over control) to soil enzyme activity than 10 FR, 5 FR, 2 FR and 1 FR treatments. Among the three different incubation times, an initial decrease was observed in all the treatments receiving the herbicides. The activities later tended to increase and in the 1 FR

Table 1. Activity of enzymes in soil as influenced by various herbicides under laboratory conditions

Herbicide conc.	Mean activity of enzymes at different concentrations											
	β-glucosidase activity (µg p-nitrophenol/g soil/h)				Protease activity (mg tyrosine/g soil/h)				Alkaline phosphatase activity (µg p-nitrophenol/g soil/h)			
	2,4-DEE	Butachlor	Pretilachlor	Pyrazo-sulfuron ethyl	2,4-DEE	Butachlor	Pretilachlor	Pyrazo-sulfuron ethyl	2,4-DEE	Butachlor	Pretilachlor	Pyrazo-sulfuron ethyl
1 FR	70.23	68.87	71.77	72.47	31.35	30.62	32.19	32.55	102.46	101.70	103.27	103.57
2 FR	68.94	67.76	70.80	71.75	30.02	29.32	31.44	32.13	101.92	100.72	102.79	103.19
5 FR	67.43	66.01	69.58	70.63	27.66	26.51	29.56	30.90	99.95	98.84	101.80	102.71
10 FR	65.69	63.71	68.75	70.05	25.08	22.77	27.51	29.13	98.69	96.56	100.99	102.24
100 FR	62.36	58.56	66.93	68.69	21.97	20.02	24.20	26.01	96.42	94.81	99.42	100.27
Control	74.43	74.43	74.44	74.43	33.54	33.54	33.54	33.54	104.87	104.87	104.86	104.85
Mean activity of enzymes at different days after application												
7 days after application	66.59	64.78	68.79	70.22	26.48	25.12	27.48	28.77	99.44	98.50	100.65	101.82
15 days after application	67.72	66.48	70.63	71.56	27.87	26.89	29.47	30.63	100.30	99.50	102.51	102.95
30 days after application	70.22	68.40	71.72	72.24	30.47	29.38	32.27	32.73	102.42	100.74	103.41	103.66
LSD (P=0.05)												
Herbicides (H)	1.325				1.119				1.335			
Concentration (C)	1.622				1.370				1.635			
Days (D)	1.147				0.969				1.156			
H x C	3.245				NS				NS			
H x D	NS				NS				NS			
D x C	NS				2.373				NS			

Initial β-glucosidase activity before herbicide application : 75.66 µg p-nitrophenol/g soil/h, Initial protease activity before herbicide application : 33.73 mg tyrosine/g soil/h, Initial alkaline phosphatase activity before herbicide application : 104.90 µg p-nitrophenol/g soil/h.
NS–Not Significant.

treatments nearly reached the values of the controls by the end of the incubation period of 30 days.

The herbicide x concentration interaction was significant for glucosidase activity. Significantly higher glucosidase activity was detected in 1 FR treatment of pretilachlor (71.77 µg p-nitrophenol/g soil/h) and in the 1 FR (72.47 µg p-nitrophenol/g soil/h) and 2 FR (71.75 µg p-nitrophenol/g soil/h) treatment of pyrazosulfuron ethyl. The days x concentration effect was significant for protease activity. Effect of herbicides on alkaline phosphatase activity was non-significant.

A decrease in the enzyme activity could be due to the fact that glucosidases, proteases and alkaline phosphatases are of extracellular origin and the death of some microorganisms could cause a reduction in the production and excretion of enzymes leading to a reduction in the soil activity (Perucci *et al*., 1999). It was observed in this experiment that the effects of herbicides towards enzyme activities decreased with time. Recovery of enzyme activities after initial inhibition could be due to growth of microbial population after adaptation or most probably due to increased availability of nutrients due to the degradation of herbicides (Ismail *et al*., 1998).

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