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Effect of herbicides butachlor, 2,4-D and oxyfluorfen on enzyme activities and CO_2 evolution in submerged paddy field soil

MANJUMANI BARUAH* and R. R. MISHRA
Microbiology laboratory, Department of Botany, School of Life Sciences, North-Eastern Hill University, Shillong-793014, India

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Summary Effects of three herbicides 2,4-D, butachlor and oxyfluorfen on activities of dehydrogenase, urease and carbon dioxide evolution from paddy field soil were measured on weekly intervals. Dehydrogenase activity was significantly stimulated on application of herbicides. The herbicides did not affect the urease activity and the activity remained almost unchanged. The carbon dioxide output was higher in herbicide treated soil.

Introduction

Any compound which alters the number or activity of microorganism could affect the soil biochemical processes[21,25] and ultimately also the soil fertility and plant growth. In recent years, herbicides have been applied to agricultural soils extensively either as pre- or post-emergence treatments to control weeds. Several studies are available on the impact of herbicides on microbial activity of dry land agricultural soils[2,4,5,7,9,11,18]. The residual effect of herbicides on submerged paddy soil enzyme activities and carbon dioxide evolution has not been thoroughly understood[2].

The present paper examines the influence of three herbicides, recommended for paddy fields, *viz.* 2,4-D (weedex) butachlor (machete) and oxyfluorfen (goal) on dehydrogenase and urease activities and carbon dioxide evolution of paddy field soils.

Materials and methods

Surface (0–15 cm) soil was collected from submerged paddy field. Some of its characteristics are given in Table 1. The most commonly used post-emergent herbicides in the paddy fields of India representing different chemical groups were selected. Those used were 2,4-D, butachlor and oxyfluorfen. The details of herbicides are given in Table 2.

Experimental details

The manufacturer's recommended doses in kg/hectare were converted to g or ml/kg soil. For these conversions certain standard assumptions were followed: (a) the herbicides were distributed

Table 1. Soil characteristics

Soil type	Sandy loam
pH	6.50
Organic carbon	2.16%
Available phosphorus	0.04%
Total nitrogen	0.30%
Exchangeable potassium	0.06%

*Department of Botany, Gauhati University, Gauhati 781014, India

Table 2. Herbicides with their chemical names, recommended doses and manufacturers

Commercial name of formulations	Chemical and common name of active ingredient	Recommended dose of commercial product	Manufacturer
Weedex	2-4-Dichlorophenoxy acetic acid (2,4-D)	19.76 kg/ha	Agromore Ltd, Calcutta
Machete	2 chloro 2′ 6′ diethyl-n-butoxy-methyl acetanilide (butachlor)	2.47 l/ha	Monsanto Ltd, New Delhi
Goal	2-chloro-1-3 ethoxy-4-nitrophenoxy-4 (trifluoro-methyl) benzene (oxyfluoren)	1.54 l/ha	Indofil Chemical Ltd, Bombay

in the 5 cm of soil, and (b) the weight of a hectare soil to a depth of 5 cm was approximately 7.3×10^5kg[13]. With this assumption an application rate of 1 kg/hectare was thereby equivalent to 1.4 mg/kg. Manufacturer's recommended doses were used in case of all the herbicides. Control soil was sprayed with an equal volume of distilled water. The final moisture content of each was adjusted to the field capacity. Soils were incubated in plastic pots at 30°C for 6 weeks[6]. The moisture content of the soil was adjusted every week with distilled water. There were three replicates of each treatment.

Enzyme and carbon dioxide evolution determination

Dehydrogenase activity was measured by incubating the soil samples at 30°C for 24 hrs treating with $CaCO_3$ and triphenyl tetrazolium chloride (TTC) and extracting with methanol as described by Casida[1]. Urease activity was assayed by the method of McGarity and Myers[17]; ammonia released as a result of urease activity was determined by the Indophenol blue method. The carbon dioxide evolution was measured by soil enclosure technique using KOH as absorbent and HCl as a titrant[14]. All analyses were done in triplicate.

Results and discussion

The peak rate of dehydrogenase activity followed a trend 2,4-D > oxyfluorfen > butachlor and the lowest values at the end of the experiment followed an order of control > butachlor > 2,4-D = oxyfluorfen.

Dehydrogenase activity increased with time during the first seven days and dropped rapidly during subsequent weeks. The decline at the later periods (35 to 42 days) was quite steep whereas in untreated soil the enzyme activity was stabilized after an initial drop (Fig. 1).

The herbicide treatment did not affect the urease activity significantly. Throughout the study period urease activity was more or less the same in all three herbicide treated soils (Fig. 2). There was no significant variation in the rate of urease activity between treated and untreated soils.

The rate of carbon dioxide evolution was always higher in the herbicide treated soils compared to the control soils (Fig. 3). Carbon dioxide evolution was higher in case of butachlor sets than 2,4-D and oxyfluorfen in the last three weeks.

Initially, the dehydrogenase activity was stimulated on application of herbicides. However, decreased dehydrogenase activity was observed during later period of incubation. Rozsypalova[18] reported that herbicides used at recommended application rates caused temporary (insignificant) changes in the dehydrogenase activity. Greaves *et al.*[7] evaluated the effects of the herbicide dalapon on dehydrogenase activity and soil respiration. According to them at normal recommended dose dalapon had little effect on soil microflora and soil fertility. Urease, being a urea degrading enzyme, and does not mediate the degradation pathway of the herbicides and probably therefore, remained

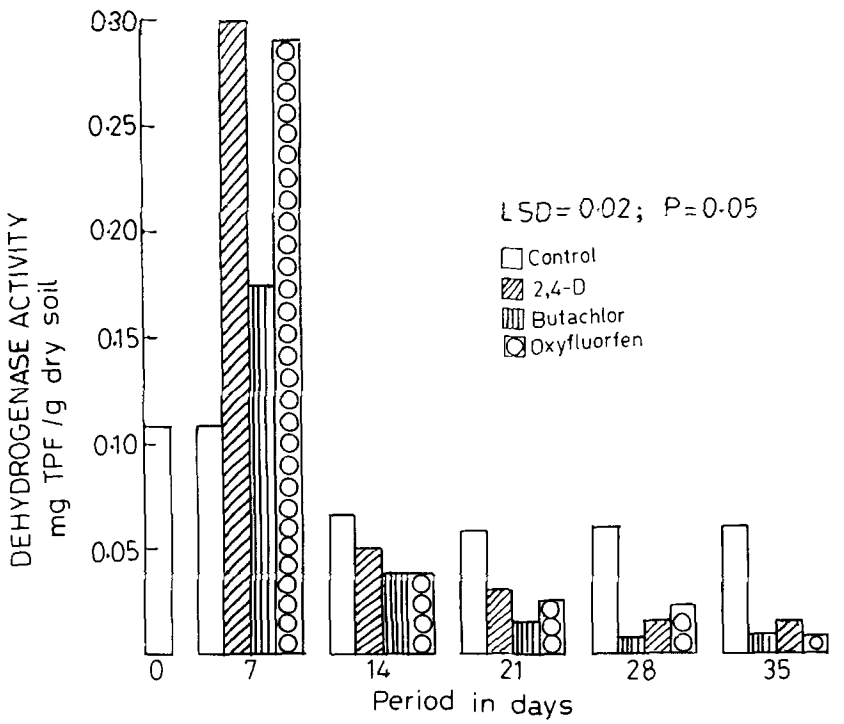

Fig. 1. Weekly variation in the dehydrogenase activity of soils treated with the herbicides, 2,4-D butachlor and oxyfluorfen.

unaffected by the herbicides (Fig. 2). Tu[21] conducted a laboratory experiment using 32 pesticides and reported that none of the pesticides inhibited urease activity. It appears that the herbicides were inert to urease producing microbes and urease activity. Similar results were reported by several other workers[3, 11, 20, 23]. Also, it shows that except the pathways through which the herbicides are degraded (possibly dehydrogenase) other microbial activities remain unaltered by the herbicides. It was significant that the urease remain unaffected by all the herbicides. Contrary to our results Lethbridge and Burns[12] and Marsh[16] detected an increase in urease activity in asulam treated soil whereas there was no effect on dehydrogenase activity.

Herbicides application increased the rate of CO_2 evolution during initial periods but it decreased significantly towards end of the experiment (Fig. 3). The CO_2 evolution rate was much as expected with the increased bacterial population and dehydrogenase activity. Davies and Marsh[4] also reported that herbicides stimulated the CO_2 evolution during early part of incubation period. Greaves *et al.*[7] reported that in soil, carbon dioxide evolution was initially increased while dehydrogenase activity declined. Helweg[10] found a reduction in CO_2 output from soil during a 15 days incubation of the 50 and 100 ppm chlorthismid treated soils. Stimulatory effect of herbicides on CO_2 output has been observed by several workers[15, 22, 25]. The increased rate of CO_2 evolution can be attributed to the degradation of herbicides. According to Grossbard and Davies[9], with normal rates of application, the herbicides appear to have little inhibitory influence on soil respiration, although few herbicides are without effect at higher concentration. The present study emphasised that the dehydrogenase activity is generally stimulated by the application of herbicides. But this does not conclude that the herbicides are beneficial or nontoxic to the soil microbes. A review of the literature suggests that results of one study using one herbicide and soil cannot be applied to any other herbicide (even in same chemical group) or soil in general. Chen *et al.*[2] indicated that machete used at recommended rate did not change the soil properties significantly. He also demonstrated that machete may not cause a serious problem of environmental pollution.

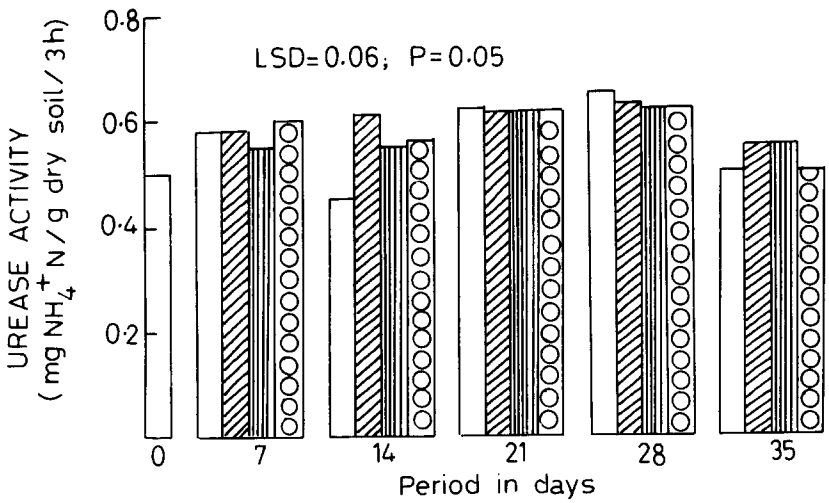

Fig. 2. Weekly variation in the urease activity of soils treated with the herbicides, 2,4-D, butachlor and oxyfluorfen. Histograms as in Fig. 1.

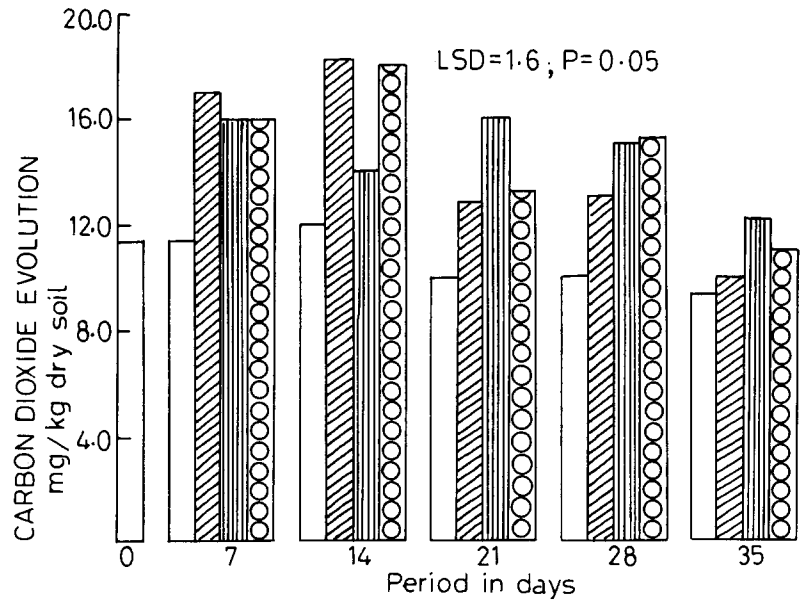

Fig. 3. Weekly variation in the carbon dioxide evolution of soils treated with the herbicides, 2,4-D, butachlor and oxyfluorfen. Histograms as in Fig. 1.

Wainwright[24] reported that common agricultural practices have more pronounced effect on soil properties than herbicides. On the other hand, some workers still find it difficult to recognise any undesirable effect of herbicide treatment, because our knowledge of how microorganisms affect soil fertility is inadequate[8,9]. Fryer *et al.*[5] and Smith[19] also concluded that the herbicides did not have any long term harmful effect on biological activity and fertility of soils when used at recommended dose.

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