Effects of Paraquat and Alachlor on Soil Microorganisms in Peat Soil

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ABSTRACT
A study was carried out to investigate the effects of alachlor and paraquat on microbial activities in peat soil. Effects of the herbicides on CO$_2$ evolution and phosphatase activity were monitored for 12 weeks in ambient conditions. The results showed that paraquat and alachlor caused an initial increase in CO$_2$ released and subsequently decreased after 53 days of incubation. Comparatively, more CO$_2$ was released from the soil treated with alachlor than that treated with paraquat. An initial increase in phosphatase activity was observed for both herbicides but the level of activity was substantially reduced after 12 days of incubation. Fungal and bacterial populations in the soil were also affected by both herbicides. At 250 ppm, alachlor and paraquat caused a reduction in bacterial population of 78% and 95% respectively. Alachlor was shown to be more toxic to fungal populations in the soil than paraquat.

INTRODUCTION
Microorganisms play important roles in soil processes, among which are the recycling of essential plant nutrients, humus formation, and pesticide detoxification. In agriculture, a major concern over the usage of herbicides is the possible harmful effects exerted on the soil microflora, which contribute to soil fertility. Herbicides are equally toxic to plants as well as to many soil microorganisms. Problems in assessing the impact of herbicides on the soil microflora are numerous and complicated. This is largely due to the highly complex nature of multiple interactions occurring simultaneously between herbicide, soil, and micro-organisms.

There are several ways of assessing the effects of herbicides on microbial activities and these include detecting CO$_2$ evolution, measurement of O$_2$ uptake, estimation of microbial population levels and assaying soil enzyme activities. Each parameter has its advantages and disadvantages. In recent years, the measurement of soil biological activity has increasingly relied on assays for soil-borne microbial enzymes such as phosphatase (Marsh 1980; Davies and Greaves 1981). The present study describes the effects of alachlor and paraquat on soil microflora.
MATERIALS AND METHODS

Soil

Peat soil was obtained from the top 0-5 cm of an uncultivated plot at MARDI Research Station, Jalan Kebun, Kelang, Selangor. The physico-chemical properties of the soil are shown in Table 1. Before use, the soil was passed through a 5 mm sieve, placed in black polythene bags and stored at 4°C.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.8</td>
</tr>
<tr>
<td>% water content</td>
<td>80</td>
</tr>
<tr>
<td>% carbon</td>
<td>34.2</td>
</tr>
<tr>
<td>% organic matter</td>
<td>59.3</td>
</tr>
<tr>
<td>% N</td>
<td>3.1</td>
</tr>
<tr>
<td>% clay</td>
<td>44.6</td>
</tr>
<tr>
<td>% silt</td>
<td>11.7</td>
</tr>
<tr>
<td>% sand</td>
<td>43.7</td>
</tr>
<tr>
<td>CEC (M equiv/100 g soil)</td>
<td>145.0</td>
</tr>
</tbody>
</table>

Herbicides

The two herbicides tested were paraquat (Gramoxone®, ICI) containing 20% w/v of 1,1'-dimethly-4,4'-bipyridylium chloride, and alachlor (Lasso®, Monsanto) containing 48% w/v of chloro 2', 6'-diethyl-N- methoxymethyl.

Soil treatment

Soil samples were treated with both alachlor and paraquat according to the commercial formulation of either Lasso® or Gramoxone®. Moist soil equivalent to 4 kg oven-dry soil was placed in a cylindrical metal drum (30 x 27.5 cm) containing a polythene liner. Each herbicide was applied separately by spraying onto the soil to give a mean final concentration of 0, 100 or 250 ppm of the active ingredient (calculated on an oven-dry basis).

The mixture was mixed thoroughly in a rotating drum. The moisture content was then adjusted to 80% of field capacity by adding deionized water as necessary.

Carbon dioxide evolution

Carbon dioxide evolution was measured using a continuous gas flow system as described by Grossbard and Marsh (1974). Two samples of 100 g of soil were taken from each replicate one day after spraying and incubated in 500 ml respiration flasks attached to a manifold distributing a slow flow of moist CO₂-free air. This was passed through the layer of soil in the flask from an inlet close to the bottom of the vessel and the CO₂ was absorbed in 40 ml M-NaOH in Drechsel bottles and measured periodically by titration with 0.05 M H₂SO₄. The soil samples were incubated at 27°C. By replacing fresh NaOH in Drechsel bottles, CO₂ evolving from the soil was recorded during 3 months' incubation. The soil moisture in the flasks was adjusted to, and maintained at, 80% field capacity.

Microbial count and phosphatase activity

Soil samples were removed from the polythene bags at day 2, 12, 32, 60 and 90 for microbial population counts and phosphatase assay.

Total counts of fungal and bacterial populations were determined by the plate count method using Potato Dextrose Agar for fungal, and nutrient agar (NA) for bacterial growth. Ringer's solution was used as diluent at all times. Plates were incubated at 30±3°C. Dilutions of soil samples were made in triplicate.

Phosphatase activity was measured on 1 g samples of soil by the method of Tabatabai and Bremner (1969). The production of p-nitrophenol was determined spectrophotometrically at 420 nm.

RESULTS AND DISCUSSION

Addition of alachlor or paraquat to soil initially increased the rate of CO₂ evolution during the first 7 days' incubation. The initial stimulation of CO₂ evolution in herbicide-treated soil may be due to direct stimulation of respiratory activity of the soil microbes. A similar observation has been reported by Quilt et al. (1979) in barban-treated soil. The rate of CO₂ released was affected in soil treated with 250 ppm alachlor during the first 50 days' incubation (Fig. 1). The CO₂ from soil treated with alachlor was higher than from control soil after 70 days of incubation. CO₂ evolution from paraquat-treated soil at 100 ppm was not significantly different from control between day 7
EFFECTS OF PARAQUAT AND A1ACHLOR ON SOIL MICROORGANISMS IN PEAT SOIL

Fig. 1: Effect of herbicides on CO$_2$ evolution from the soil. ■ control, ● 100 ppm alachlor, ▲ 250 ppm alachlor, ◇ 100 ppm paraquat, △ 250 ppm paraquat.

until day 50 but thereafter it showed significantly higher readings than control. The higher evolution of CO$_2$ at day 60 may be due to an increase of microbial activity. At certain concentrations, paraquat may serve as a possible carbon and/or nitrogen source for certain bacteria and fungi (Camper et al. 1973). But at higher doses, paraquat inhibited growth and activity of certain fungi (Tu and Bollen 1968). Therefore, CO$_2$ evolution from soil treated with 250 ppm paraquat did not show an increment after 7 days of incubation.

The amount of CO$_2$ released is correlated with the presence of different species of microbes in the soil. The populations of soil fungi and bacteria were affected by treatment with either alachlor or paraquat (Fig. 2). An increase of paraquat concentration was less affective on fungal count in soil. Paraquat treatment appeared to cause a greater reduction of bacterial than fungal counts. In previous studies, two fungal species, Aspergillus sp. and Penicillium sp. were found to be dominant in soil treated with paraquat and they have been reported to be paraquat-resistant (Smith et al. 1976). The behaviour of paraquat in soil may explain the differences between the two herbicides. Paraquat was also found to be readily absorbed by soil particles through its ionic exchange capacity (Weber and Coble 1968). The adsorption of paraquat molecules onto soil particles may reduce their deleterious effects on soil microbes (Weber and Coble 1968). In contrast, alachlor is soil-active and this may be the factor contributing to the reduction of both fungi and bacterial populations. At a higher concentration (250 ppm), alachlor caused 81% reduction of the fungal population, whereas paraquat resulted in only 29% reduction.

From the data reported here, it is evident that paraquat and alachlor are equally toxic to bacteria and to fungi. The bacterial population in untreated soil was approximately $7.5 \times 10^4$/g dry soil. At 250 ppm, alachlor and paraquat caused 78% and 75% reduction, respectively in the bacterial and fungal populations (Fig. 2). Since microbial flora contribute significantly toward the improvement of the soil, these effects on bacteria and fungi could give a negative response to the usage of herbicides.

Phosphatase activity was generally very low in control and treated soil during 7 days of incubation but reached a maximum at day 10. The level of phosphatase in soil treated with 100 ppm paraquat was higher than the activity in alachlor-treated soil at the same concentration, 32 days after spraying. Consequently, phosphatase activity was still higher in soil treated with paraquat as compared to alachlor-treated soil when incubation period was prolonged to 60 days. The difference in behaviour between the two herbicides in soil explained the differences in phosphatase activity. Phosphatase activity in soil treated with either herbicide at 92 days showed correlation with microbial population in which lower microbial population was observed at the higher herbicide concentrations. Earlier reports have shown that herbicides may enhance or inhibit soil enzyme activities (Quilt et al. 1979; Davies and Greaves 1981). For instance, Quilt et al. (1979) showed that after an initial inhibition, there was a consistent increase in phosphatase activity in soil treated with barban at 200 ppm. In contrast, atrazine significantly reduces soil enzymatic activity (Voets...
Under field conditions, it was suggested that the reduction of soil enzyme activity results partially from the indirect effect of the herbicide treatment, namely the elimination of the direct vegetative cover and the concomitant decrease in the soil organic matter (Voets et al. 1974). Organic content and other factors have an effect on soil microbe populations (Marsh et al. 1978). Therefore, reduction in phosphatase activity was also observed in control soil when incubation periods were prolonged.

The concentrations of herbicides used in this study were higher than normal application rates applied in the field, which are less than 10 ppm for the top 5 cm of soil. Therefore, the low concentration of paraquat or alachlor, which is characteristic of normal field applications, is unlikely to have any effect of agronomic importance. However, it is possible that normal application in the field may lead to uneven distribution and thus localized high concentrations of herbicide. How far these may influence plant growth and crop production will depend on how the specific microbial activity affected is related to soil fertility.

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REFERENCES


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