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Effects of Alachlor and Metolachlor on Microbial Populations in the Soil

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ABSTRACT

A study of the impact of two acetanilide herbicides, viz. alachlor and metolachlor on bacterial and fungal populations and biomass in the Sungai Buluh soil series samples was carried out under laboratory conditions. The effects of the two herbicides were monitored for 70 days under ambient conditions. Metolachlor caused greater reduction in bacterial counts than on fungal populations. There was approximately 75% reduction in bacterial counts 14 days after treatment (DAT) with 2 µg/g metolachlor. Alachlor however was less toxic to bacterial and fungal populations. Alachlor caused a reduction in bacterial counts at 7 and 14 DAT with 2µg/g or above. Fungal population decreased significantly in the presence of 20 µg/g alachlor at 7 DAT but no further effects were observed as the incubation period was prolonged. The study showed that the microbial biomass immediately decreased significantly in the presence of 2 µg/g or more of metolachlor at 0 and 28 DAT. Alachlor, on the other hands, at the lowest experimental dose of 2 µg/g reduced the microbial biomass almost immediately upon incubation, but had no further effects when the incubation period was prolonged.

Keywords: acetanilide herbicides, alachlor, metolachlor, Sungai Buluh soil

INTRODUCTION

Organic matter decomposition through microbial processes is one of the most important life processes occurring in the soil. The microflora responsible for this degradation are crucial because their activity determine the level of accumulation of plant debris on the soil surface. One of the side effects of herbicide application is the delay in pesticide decomposition, an important process in the degradation of organic compounds in general. Such delays in pesticide decomposition have the potential for exacerbating the deleterious effects of the continued presence of pesticides in the soil on the microflora responsible for the organic matter decomposition (Anderson and Domsch 1980 1989).

The acetanilide herbicides alachlor and metolachlor are registered for control of most annual grasses and certain broadleaved weeds in many crops such as maize (*Zea mays* L.), soybean (*Glycine max* [L.] Merr.), and peanut (*Arachis hypogaea* L.) (Ramesh and Maheswari 2004). In Malaysia, alachlor and metolachor are used for weed control in peanut and maize crops.

Acetanilide herbicides are degraded quickly by soil microbes; their half-lives are relatively short. The dissipation of alachlor in the soil was found to follow the first-order kinetics and the half-life in the soil was reported to be 7.8 days (as conducted by Beestman and Deming 1974). However, Zimdahl and Clark (1982) reported that the half-lives of alachlor and metolachlor at 50% field capacity were 8 and 12 days, respectively. The persistence of these herbicides is dependent on many factors such as temperature, soil moisture, pH and nutrient level (Beestman and Deming 1974; Taiwo and Oso, 1997). Their effects on microbial populations in Malaysian soils have frequently been reported, unlike those of other herbicides such as paraquat (Ismail *et al.* 1992) and metsulfuron-methyl (Ismail *et al.* 1996). Microbial degradation is one of the important processes involved in reducing alachlor and metolachlor in the soil (Stamper and Tuovinen 1998). These compounds could be degraded under aerobic and unaerobic conditions (Konopka 1994). The majority of the studies reporting the side effects of pesticides on soil fungi and other soil inhabiting microorganisms have been carried out in temperate regions. Sette *et al.* (2004) reported that *Streptomyces* was one of the microbes which was able to degrade and detoxify alachlor residues in the soil. Limited information has been published on the effects of acetanilide herbicides such as alachlor and metolachlor on the soil microflora in tropical soils (Gupta and Moolani 1970). Sharma (2002) who studied the degradation of alachlor in water and tropical soils of India reported that no residue was detected in the soils 80 DAT.

The purpose of the present study was to assess the influence of alachlor and metolachlor on microbial populations and biomass in agricultural soils of the Sungai Buluh series in Malaysia.

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MATERIALS AND METHODS

Soil Sample

The soil samples of the Sungai Buluh Series (86.3% sand, 3.7% silt, 10% clay, 2% organic carbon and pH 4.80) were collected from the top 5 cm of soil at the rubber estate of the Rubber Research Institute of Malaysia, located at Sungai Buluh, Selangor.

Prior to soil treatment, the air-dried soil was passed through a 2-mm sieve, then placed in black polythene bags and stored at - 4°C. The herbicides tested were: metolachlor (2-chloro-N-[2-ethylphenyl]-N-[2-methoxy-1-1methylethyl]acetamide) (Dual®, Ciba Geigy containing 720 g a.i./L) and alachlor (2-chloro-N-(2,6-diethyl phenyl)-N-(methoxymethyl) (Lasso®, Monsanto containing 480 g a.i./L.

Soil Treatment

The equivalent of 3 kg of air-dried soil was placed in a cylindrical metal drum (30 cm x 27.5 cm) lined with a polythene sheet. The herbicide, either alachlor or metolachlor was applied by spraying onto the soil samples to give a mean final concentration of 0, 2, 20 and 40 µg/g of the active ingredient (calculated on air-dried basis). The treated soil was mixed thoroughly in rotating drum. The untreated soil was used as control. The soil moisture content was then adjusted to 50% field capacity by adding the appropriate amount of water. Three replicate consisting of 3-kg soil samples per treatment were placed in double black polythene bags and kept at 22°C. The bags were opened once a week to prevent the soil becoming oxygen-deficient. The soil moisture levels were checked regularly by weighing and were adjusted to 50% field capacity by adding the necessary quantity of deionized water.

Bacterial and Fungal Populations

Total counts of fungal and bacterial propagules in the soil samples were made by the plate count method as described by Ismail and Yap (1994). Soil was taken from each bag and mixed thoroughly. The first samples were taken immediately after treatment (referred to as day 0), and followed up at days 7, 14, 21, 28, 35, 42, 49, 56 and 70 for the microbial enumeration study. Soil suspensions were prepared by homogenizing 5 g of soil in 50 ml of quarter-strength Ringer solution for 30 min at 200 rpm. A series of ten-fold dilutions between 10^{-1} and 10^{-5} of the suspension was made with sterile Ringer solution. Each dilution was gently agitated throughout the plating procedure for 15 min. A preliminary experiment showed the 10^{-3} dilution to be suitable for the study, so it was used throughout the process for enumerating bacterial populations. For bacterial counts, ca. 0.1 ml of this suspension was transferred to each of five Petri dishes containing nutrient agar with the addition of nystatin and cyclohexamide at 50 µg/ml each for suppression of fungal growth. For fungal counts, the above dilutions were plated onto potato dextrose agar to which was added streptomycin (30 µg/ml) and Rose Bengal (50 µg/ml) to suppress bacterial growth. All dishes were incubated at 27°C, following which the colonies of fungi and bacteria were counted after 3 days' incubation.

Soil Microbial Biomass

The procedures for measuring soil microbial biomass were similar to the basal respiration measurement procedure (CO_2 evolution) as described by Ismail and Kassim (1994). Glucose was added so that the physiologically-active microbial biomass could be determined (Anderson and Domsch 1980). Preliminary experiments have shown that 6 mg glucose/gm soil (on a dry weight basis) was needed for biomass measurement, since this was the minimum amount required to provide maximal initial CO_2 evolution by the microbial biomass following substrate addition. This quantity of glucose was thoroughly mixed into 50 g of soil, and replicated five times. Results were described in terms of the rate of CO_2 evolution, 4 hr after the glucose addition at 22°C. The maximum initial rates of respiration were determined and the values obtained were tabulated into the equation $x = 40.04\ y + 0.37$, where x = mg microbial C per unit soil and y = ml CO_2 per unit soil per hr with mg CO_2 per day = 0.021 ml CO_2 per hr (22°C; 101.3 kPa) (Anderson *et al.* 1981).

RESULTS AND DISCUSSION

Table 1 shows the effects of metolachlor on microbial populations in the Sungai Buluh soil series samples. The bacterial populations decreased significantly in the presence of 2 µg/g metolachlor at 7, 14, 21 and 35 days after treatment (DAT). Greater reduction (approx. 75%) of bacterial counts was observed in the presence of 2 µg/g metolachlor at 14 DAT. A significant reduction in bacterial populations was also observed as the concentration of metolachlor increased to 40 µg/g on day 7 until 35 DAT. However at 42 DAT, the population had recovered and was comparable to the control. In general the fungal population in the control and treated soil decreased as the incubation period was prolonged. Fungal populations decreased in the presence of 2, 20 or 40 µg/g metolachlor during the first 7 DAT. However, the population recovered by day 14 of the incubation period even at the highest concentration applied. With prolonged incubation, metolachlor did not affect the fungal populations, irrespective of the concentrations used.

Table 2 shows the microbial populations in the Sungai Buluh soil series samples treated with alachlor. Bacterial populations decreased in the presence of alachlor at concentration 2 µg/g at 7 and 14 DAT. With prolonged incubation however, alachlor had no further effect on the bacterial populations except at 42 – 56 DAT. An increase in alachlor concentration to 20 and 40 µg/g showed a decrease in bacterial population at 7 and 14 DAT but the population of bacteria increased later on

Table 1: Mean microbial population counts in the Sungai Buluh soils series samples treated with metolachlor at various concentrations

Concentration (µg/g)	Bacteria (x 10^4 / g of soil)									
	Days after treatment									
	0	7	14	21	28	35	42	49	56	70
Control	5.0^b	4.0^a	8.0^a	6.0^a	4.0^a	6.0^a	5.0^a	5.0^b	4.0^a	3.0^a
2	6.0^a	2.0^b	2.0^d	4.0^b	4.0^a	4.0^b	5.0^a	7.0^a	4.0^a	3.0^a
20	5.0^b	4.0^a	4.0^b	4.0^b	4.0^a	4.0^b	3.0^b	5.0^b	4.0^a	2.0^b
40	4.0^b	3.0^b	3.0^c	5.0^a	3.0^b	4.0^b	5.0^a	4.0^b	4.0^a	3.0^a
	Fungi (x 10^2 / g of soil)									
Concentration (µg/g)	Days after treatment									
	0	7	14	21	28	35	42	49	56	70
Control	4.0^a	4.0^a	2.0^c	2.0^b	4.0^{ab}	3.0^b	2.0^b	2.0^c	2.0^b	1.0^b
2	3.0^b	3.0^b	3.0^b	4.0^a	3.0^b	2.0^c	3.0^b	4.0^a	2.0^b	1.0^b
20	3.0^a	3.0^b	3.0^b	4.0^a	4.0^a	7.0^a	4.0^a	3.0^b	2.0^b	2.0^a
40	3.0^b	3.0^b	4.0^b	4.0^a	3.0^b	3.0^b	3.0^b	2.0^c	4.0^a	2.0^a

Means within a column followed by the same alphabet are not significantly different according to Duncan's multiple range test at the 5% level.

Table 2: Mean microbial population counts in the Sungai Buluh soils series samples treated with alachlor at various concentrations

Concentration (µg/g)	Bacteria (x 10^4 / g of soil)									
	Days after treatment									
	0	7	14	21	28	35	42	49	56	70
Control	8.0^a	7.0^a	7.0^a	5.0^a	4.0^a	4.0^a	4.0^a	3.0^a	2.0^a	5.0^a
2	7.0^a	5.0^b	5.0^b	5.0^a	5.0^a	4.0^{ab}	3.0^a	1.0^b	1.0^d	5.0^{ab}
20	7.0^a	6.0^b	5.0^b	4.0^a	4.0^a	3.0^b	3.0^a	2.0^a	1.0^c	4.0^b
40	7.0^a	6.0^b	5.0^b	5.0^a	4.0^a	3.0^c	3.0^a	2.0^a	2.0^b	6.0^a

Concentration (µg/g)	Fungi (x 10^2 / g of soil)									
	Days after treatment									
	0	7	14	21	28	35	42	49	56	70
Control	2.0[a]	3.0[a]	2.0[a]	2.0[a]	1.0[a]	2.0[a]	2.0[b]	2.0[a]	2.0[a]	2.0[a]
2	2.0[a]	3.0[a]	2.0[a]	2.0[a]	1.0[a]	2.0[a]	3.0[a]	2.0[a]	2.0[a]	2.0[a]
20	1.0[a]	2.0[b]	2.0[a]	2.0[a]	1.0[a]	1.0[b]	2.0[b]	2.0[a]	2.0[a]	1.0[a]
40	1.0[a]	2.0[b]	3.0[a]	2.0[a]	1.0[a]	1.0[b]	1.0[c]	2.0[a]	2.0[a]	1.0[a]

Means within a column followed by the same alphabet are not significantly different according to Duncan's multiple range test at the 5% level.

Table 3: Estimated microbial biomass (µg microbial/g of soil) in the Sungai Buluh soils series samples after treatment with either metolachlor or alachlor at various concentrations

Concentration (µg/g)	Metolachlor					
	Days after treatment					
	0	14	28	42	56	70
Control	404.40[a]	324.02[a]	282.48[a]	223.92[a]	203.90[a]	63.76[b]
2	364.06[b]	304.00[a]	203.90[b]	183.88[a]	183.88[ab]	43.81[b]
20	324.02[b]	223.92[b]	203.90[b]	183.88[a]	183.88[ab]	103.80[a]
40	283.98[b]	223.92[b]	183.88[b]	203.90[a]	123.82[b]	103.80[a]
	Alachlor					
Concentration (µg/g)	Days after treatment					
	0	14	28	42	56	70
Control	404.40[a]	223.92[b]	183.88[b]	223.92[a]	203.90[a]	63.76[a]
2	364.06[b]	243.94[b]	203.90[ab]	103.80[c]	143.84[a]	123.82[a]
20	384.08[b]	223.92[b]	163.86[b]	283.98[a]	143.84[a]	103.80[a]
40	364.06[b]	344.04[a]	263.96[a]	223.93[b]	163.86[a]	83.78[a]

Means within a column followed by the same letter are not significantly different to Duncan's multiple range test at the 5% level.

except on day 56 and 70 at the concentration of 20 µg/g, respectively. The fungal population decreased significantly in the presence of 20 µg/g alachlor at 7 DAT. Increasing the concentration of alachlor to 40µg/g had no further effect on the fungal populations in the soil samples tested. Further incubation showed that increasing the alachlor concentration did not affect the fungal populations in the test soil.

Table 3 shows the effects of metolachlor and alachlor on the estimated microbial biomass in the Sungai Buluh soil. An increase of metolachlor concentration significantly affected microbial biomass at 0 DAT (i.e. immediately after treatment) and at 28 DAT in the presence of 2 µg/g metolachlor. A significant reduction of the biomass was observed at 14 DAT in the presence of higher concentration (20 µg/g) of metolachlor. As in soil treated with metolachlor, the microbial biomass decreased as the incubation periods with alachlor were prolonged, irrespective of the concentration applied. Similarly, a significant reduction in the microbial biomass was observed immediately after treatment in the presence of 2, 20 and 40 µg/g alachlor. During other incubation periods, it seems that increasing the alachlor concentration did not affect the microbial biomass except on day 14 in soil treated at 40 µg/g. The microbial biomass increased at 14 and 28 DAT in soil treated with alachlor at 40 µg/g.

The results of this study showed that both acetanilide herbicides, metolachlor and alachlor have an inhibitory effect on the microbial population and its biomass in the soils. The results clearly showed that the different characteristics of the two herbicides have a marked effect on microbial populations. In soil, metolachlor was more persistent than alachlor (Zimdahl and Clark 1982), and was also slightly but significantly more toxic to microbes and plants (barnyardgrass) than alachlor (Weber and Peter 1982). The present results showed that metolachlor caused greater reduction in the microbial populations present in the soil studied as compared to alachlor especially with regard to bacterial counts. On the other hand, alachlor is less persistent and more readily degraded by soil microorganisms, which reduce their residual activity (Smith and Phillips 1975; Stamper and Tuovinen 1998). Alachlor is also readily adsorbed on to soils with higher clay and organic matter content, and this may reduce its effect on soil microorganisms (Rahman *et al.* 1978; El-Nahhal 2003). Therefore, alachlor was found to be less toxic to soil microbes than metolachlor.

The concentrations of herbicides used in this study were higher than normal rates applied in the field, which are less than 10 µg/g for the top 5 cm of soil. Therefore, the low concentration of metolachlor or alachlor, in the soil when applied at the normal field application rates is most unlikely to cause any detrimental effect on soil microbes and soil fertility. However, it is possible that even at a normal application rate in the field there could be uneven distribution, thus causing localized area of high concentration of the herbicide. However, the influence on plant growth and crop production will depend on the specific microbial activity affected in relation to soil fertility. Previous studies have shown that the half-life of metolachlor in the soil was 12 days (Zimdahl and Clark 1982). It should be noted that the present results are obtained from a laboratory study, whereby the environmental factors were controlled. Under field conditions, the soils are exposed to wind, rain, fluctuations in temperature etc. Therefore, it is suggested that the study on the effects of these herbicides should be carried out under natural conditions in order to obtain a clearer picture of their effects on soil microorganisms.

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