African Journal of Microbiology Research Vol. 7(5), pp. 367-374, 29 January, 2013
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.1277
ISSN 1996 0808 ©2013 Academic Journals

Full Length Research Paper

Effects of selected herbicides on soil microbial populations in oil palm plantation of Malaysia: A microcosm experiment

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Accepted 9 January, 2013

Herbicides are commonly used in Malaysia to control weeds in oil palm plantation. In addition to their impact on weeds, these herbicides are also affecting soil microorganisms which are responsible for numerous biological processes essential for crop production. In the present study, we assessed the impact of four commonly used herbicides (paraquat, glyphosate, glufosinate-ammonium and metsulfuron-methyl) on soil microbial populations in oil palm plantation. Our study showed that the herbicide treatments significantly inhibited the development of microbial populations in the soil, and the degree of inhibition closely related to the rates of their applications and varied with the types of herbicide. Paraquat caused the highest inhibitory effect to bacteria and actinomycetes, whereas fungi were most affected by glyphosate. Metsulfuron-methyl had least inhibitory effects to all the microbial populations. The highest inhibition (59.3%) for fungal population was observed at 6 DAT (days after treatment), whereas for the bacteria and actinomycetes (82.0 and 70.6%, respectively) were at 4 DAT. Increasing trend of inhibition on growth of microbial populations was observed from the initial effect until 6 DAT, followed by a drastic decrease of the inhibition at 10 DAT. No inhibition was observed at 20 DAT. The study suggests that the herbicide application to soil of oil palm plantation cause transient impacts on microbial population growth, when applied at recommended or even as high as double (2x) of the recommended field application rate.

Key words: Herbicides, soil microbes, soil microcosm, field application rate, oil palm plantation.

INTRODUCTION

Soil, an important component of the ecosystem, serves as a medium for plant growth through the activity of microbial communities. This soil microbial communities (like bacteria, fungi and actinomycetes) play critical role in litter decomposition and nutrient cycling, which in turn, affect soil fertility and plant growth (Singh et al., 1999; Chauhan et al., 2006; Tripathi et al., 2006; Pandey et al., 2007). However, soil micro-organisms are greatly influenced by factors including the application of herbicides (Pampulha et al., 2007), which are applied in modern agricultural practices to attain optimum crop yields (Zabaloy et al., 2008). If, microorganisms are sensitive to particular herbicide, its application will interfere with vital metabolic activities of microbes (Oliveira and Pampulha, 2006), thus affect the availability of nutrients in the soil (Nautiyal, 2006). Numerous studies have shown the effect of herbicides on soil micro-organism populations that ultimately affect the rates of decomposing labile, celluloses and recalcitrant like lignin, respectively, in a variety of ecosystems (Taylor and

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Parkinson, 1988; Tripathi and Singh, 1992a,b; Pandey et al., 2007; Osono et al., 2003; Osono and Takeda, 2007; Osono et al., 2008). Although, their accurate numbers are still not very clear mainly because of rapid changes in the populations (Chauhan et al., 2006; Das et al., 2006), but a healthy population of microorganisms can stabilize the ecological system in soil (Chauhan et al., 2006). Thus, the changes in the population of these micro-organisms will affect the ability of the soil to regenerate nutrients to support plant growth.

Malaysia is the world's largest producer and exporter of palm oil that covers over 5 million hectares of land (MPOB, 2011). Weed management is a major problem in the oil palm plantation during the immature phase to avoid suppression of growth and late yield of the oil palm (Chee et al., 1992), so the herbicides are frequently used to manage weeds. Most commonly used herbicides are paraquat, glufosinate-ammonium, glyphosate and metsulfuron-methyl (Chuah et al., 2005; Kuntom et al., 2007). The presence of herbicide residues in soil could have direct impacts on soil microorganisms is matter of great concern. At normal field recommended rates, herbicides are considered to have no major or long-term effect on microbial populations (Audus, 1964; Bollen, 1961; Fletcher, 1960). It has been reported that some microorganisms were able to degrade the herbicide, while some others were adversely affected depending on the application rates and the type of herbicide used (Wilkinson and Lucas, 1969; Sebiomo et al., 2011). Therefore, effects of herbicides on microbial growth, either stimulating or depressive, depend on the chemicals (type and concentration), microbial species and environmental conditions (Bollen, 1961; Hattori, 1973).

Studies on pesticide residual effects on soil microorganisms are often done in soil microcosm small-scale experiment which can be interpreted accurately at larger scales (Benton et al., 2007). Microcosms containing soil microfauna of field communities offer higher resolution of ecotoxicological effects of chemicals in soil environments (Parmelee et al., 1993). As the precise assessment of the potential non-target effects of herbicides on soil microorganisms in oil palm plantation are of growing interest, therefore, soil microcosm can provide better understanding of possible response of soil microbes to herbicides. The study was aimed to evaluate the effect of commonly used herbicide on bacterial, fungal and actinomycetes populations in soil microcosms from oil palm plantation.

MATERIALS AND METHODS

Herbicide treatments

The herbicide treatments consisted of paraquat (Gramoxone® PP910), glyphosate (Roundup®), glufosinate-ammonium (Basta 15®) and metsulfuron-methyl (Ally® 20 DF). Three different concentrations (rates) of each herbicide treatment: paraquat and glufosinate-ammonium at 0.44, 0.88 and 1.76 mg a.i./g soil each; glyphosate at 0.88, 1.76 and 3.52 mg a.i./g soil; and metsulfuron-methyl at 0.015, 0.03 and 0.06 mg a.i./g soil were applied in this study. These treatment rates represented 0.5, 1 and 2 times (x) their recommended field rates (paraquat: 400 g a.i./ha; glufosinate-ammonium: 400 g a.i./ha; glyphosate: 800 g a.i./ha; metsulfuron-methyl: 15 g a.i./ha). The treatments were calculated using the formula:

$$\text{X mg/g soil} = \frac{\text{Recommended field application rate (g a.i./ha)}}{\text{Amount of a.i. in formulation (g a.i./L) X 450 L/ha}} \text{X} \frac{1000\ \text{mg}}{1\ \text{g}}$$

Soil sampling and preparation of microcosm

Soils were collected from a young oil palm (3 years old) area at Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. The site has a history of herbicide application at 6-months interval, and the herbicide used is glyphosate (Roundup®). Eighty soil cores (approximately 40 kg) were sampled to a depth of 15 cm using auger, collected randomly from underneath the surrounding palms and between the palm rows. The samples were mixed thoroughly to form a composite sample and taken back to Microbiology Laboratory, Department of Plant Protection, Faculty of Agriculture, UPM, and processed accordingly. The pH value of sampled soil was determined as 4.1 ± 0.01. Soil chemical properties were determined which were as follows: 1.94% C, 0.32% N; 219 ppm P, 104 ppm K, 119 ppm Ca and 32 ppm Mg, and the soil was classified as sandy clay (40% clay, 10% silt and 50% sand).

The microcosms were prepared according to Oliveira and Pampulha (2006) with minor modifications. The soils were air-dried slowly in laboratory environment (25°C; 50% RH) for 24 h before sieving through a 2 mm mesh. The sieved soils were then analyzed to estimate the moisture content and the Water Holding Capacity (WHC). The laboratory determination of the moisture content of soil samples was done by placing 10 g of soil sample in a weighing glass beaker was initially weighed, followed by oven drying at 70°C for 24 h. Glass beaker containing the dried soil was then weighed again to get the final weight of the soil. The moisture content was calculated as percentage using the formula:

$$\text{Moisture content} = \frac{(\text{Weight of moist soil} - \text{Weight of dried soil})}{\text{Weight of dried soil}} \text{X } 100$$

Water Holding Capacity (WHC) of the soil was determined by placing 3 g of soil sample on a piece of Whatman filter paper which had been initially weighed, followed by oven drying at 70°C for 24 h. Oven-dried soil on the weighed Whatman filter paper was weighed before dipping into water until the soil was saturated. The soil was then placed in humid enclosure to drain off the water before weighing again, and calculated using the formula (ASTM, 2010):

$$\text{Water Holding Capacity (WHC)} = \frac{\text{Mass of water contained in saturated soil}}{\text{Mass of saturated soil}} \text{X100}$$

The bulked soils with determined moisture content of 13% were then mixed together, and 56 ml sterile distilled water was added to achieve the moisture level of 18.5%, which was 50% of its maximum MHC. The soil was then placed in 39 sterile glass bottles, each containing 1 kg of soil. Each bottle was loosely fit with cap to allow gas exchange. The soil-containing glass bottles were then incubated in dark, in a 25°C incubator, for 10 days to allow time for adaptation of microorganisms before treatment with the herbicides. The herbicide treatments were applied with the following procedures, conducted aseptically under laminar flow unless stated

otherwise. 50 ml of each herbicide treatments were sprayed to 36 out of 39 glass bottle accordingly, using hand sprayer. The herbicide was mixed thoroughly by constant shaking for 5 min. The remaining 3 glass bottle soils were served as control, and sprayed with 50 ml sterile distilled water. The soil microcosms were then formed by transferring the treated soils into each sterile square plastic container (15 cm x 15 cm x 7.4 cm) with lids loosely fitted. The soil microcosms were then incubated in darkness at 25°C. Sterile distilled water was added on weekly basis to restore the initial weight of each microcosm, maintaining the constant moisture content.

Enumeration of microbial population

Enumeration of the microbial populations was done using specific media for each microorganism. Three different growth media supplemented with inhibitors were prepared: Potatoe Dextrose Agar (PDA, Difco) supplemented with 30 mg/L streptomycin sulphate (Sigma-Aldrich) for enumeration of fungi; Nutrient Agar (NA, Oxoid) supplemented with 0.1g/L cyclohexamide (Merck) for enumeration of bacteria; and Actinomycetes Isolation Agar (AIA, Difco) supplemented with 0.5 g/L cyclohexamide (Merck) for enumeration of actinomycetes (Araujo et al., 2003). The inhibitors were added into sterilized media (121°C, 15 min) accordingly, and mixed thoroughly on hotplate and stirrer (Jenway) before pouring into each Petri dish, marked at the bottom dividing it into three sections.

Soil was collected from each microcosm at 2, 4, 6, 10 and 20 DAT (days after treatment) to assess the herbicidal effect on the microbial populations present in the soil. Five sub-samples were collected randomly from each microcosm treatment using sterile cork borer (10 mm diameter). Sub-samples from each microcosm were mixed together, and 1 g of the soil was taken to make a serial dilution. Serial dilutions were made aseptically under laminar flow by suspending the soil in 9 ml of sterile distilled water in a test tube and vortexed using vortex mixer (Vision Scientific) for 30 s to thoroughly mix them. This process was repeated until the dilutions were made up to 10^{-5} to complete the serial dilutions.

The drop plate method, conducted under sterile condition, was used for enumeration of the colonies. The test tubes of the serial dilutions were vortexed before five drops (10 µL $drop^{-1}$) of the suspension were pipetted out onto each particular section of the media (marked by dividing lines) according to dilution value of the suspensions. Dilutions selected for plating on PDA were 10^{-2} to 10^{-4} (for culturing fungi), whereas, NA and AIA were plated with the dilutions of the 10^{-3} to 10^{-5} (for culturing bacteria and actinomycetes, respectively). The plates were prepared in triplicates, covered and allowed to dry. After 1 h, the plates were inverted, sealed with parafilm to avoid contamination and incubated in darkness at 25°C.

Enumeration of colonies for bacteria, fungi and actinomycetes were done using the Colony Counter (Rocker) after 24 h, 7 and 10 days, respectively. The total up of the colonies was used to calculate the Colony-forming unit (CFU)/g dry weight of soil. Dry weight of soil was determined after oven drying at 70°C for 24 h using the formula:

Dry weight of soil = (weight of moist soil) X (1% moisture soil sample/100), and the CFU was calculated using the formula:

$$\text{CFU/g dry weight of soil} = \frac{\text{Colony} - \text{forming unit X dilution factor}}{\text{Amount of aliquot X dry weight of soil (g)}}$$

Data analysis

The experiment was conducted by Complete Randomized Design (CRD) with three replicates. Data were expressed as inhibition percentages relative to the control, and analyzed following 2-way Analysis of Variance (ANOVA) between herbicides and each exposure dates. Means were compared using Duncan's Multiple Range Test (DMRT) at $P<0.05$ using Statistical Analysis System (SAS).

RESULTS

The effect of herbicide treatments on soil microbial population was determined based on the inhibition percentages of the growth of fungal, bacterial and actinomycetes colonies in each treatment media. The growth inhibition showed an increasing trend with increased herbicide concentrations, and the microbial population showed different degree of sensitivity to the herbicide compounds at different sampling dates (exposure periods). The inhibition percentages of fungal colony development by the herbicides relative to the control (without herbicide treatment) were shown in Table 1. The inhibition percentage of fungi increased with higher application rates of each herbicide. Highest inhibitions of 63.1 to 81.4% were observed at 2x the recommended field application rate. At 0.5x the recommended field application rate, the herbicides inhibited fungal development by 42.2 to 54.1%. At recommended field application rate, these herbicides could be considered as only moderately toxic to the fungal colony development, causing moderate inhibition of 54 to 59.3%. This indicated that applications of the herbicides even at lower than the recommended field rates could be moderately detrimental to the fungal development in soil.

Moderately high inhibition percentages of the fungal colony development of more than 44% were observed within 2 DAT for the herbicides, except for paraquat. Paraquat, however, caused significantly lower inhibition (25.8%) at 2 DAT. The highest inhibition for paraquat of 54.3% was observed at 6 DAT, but was statistically insignificant compared with the inhibition rate of glyphosate and glufosinate-ammonium. Inhibition observed for glyphosate and glufosinate-ammonium were comparable at specific rates of application and times of sampling. Subsequently, the inhibition percentages of the fungal colony development at recommended field rate were insignificant among the herbicides from 6 DAT onwards. Inhibition of the fungal colony development was abruptly low for all the treatments at 10 DAT, ranging from 2.3 to 10.6%. The fungal colonies, therefore, showed their ability to recover from the toxic effect by 10 DAT, and at 20 DAT, no further inhibition or full colony recovery was observed.

Bacterial population development in soil was also affected significantly until 10 DAT by paraquat, glyphosate, glufosinate-ammonium and metsulfuron-methyl. The percentages of inhibition of the bacterial

Table 1. Effect of herbicide treatments on soil fungal population at five exposure periods in soil microcosm.

Herbicide Treatments (mg a.i./gm)		% Population inhibition relative to control (Mean ± SE)				
	RFR	**2 DAT**	**4 DAT**	**6 DAT**	**10 DAT**	**20 DAT**
Paraquat						
0.44	0.5x	$14.8^{ef} \pm 2.7$	$23.3^{g} \pm 5.1$	$45.2^{cd} \pm 3.3$	$0.2^{e} \pm 4.2$	$0.0^{a} \pm 0.0$
0.88	1x	$25.8^{e} \pm 1.8$	$33.0^{fg} \pm 1.4$	$54.3^{bcd} \pm 5.0$	$2.3^{cde} \pm 0.7$	$0.0^{a} \pm 0.0$
1.76	2x	$44.2^{cd} \pm 1.4$	$59.1^{bc} \pm 6.0$	$63.1^{abc} \pm 2.9$	$12.4^{bcd} \pm 1.5$	$0.0^{a} \pm 0.0$
Glyphosate						
0.88	0.5x	$37.9^{d} \pm 4.9$	$40.6^{def} \pm 4.9$	$54.1^{bcd} \pm 6.4$	$9.7^{bcde} \pm 1.2$	$0.0^{a} \pm 0.0$
1.76	1x	$44.0^{cd} \pm 0.9$	$46.1^{cdef} \pm 2.1$	$59.3^{abc} \pm 4.5$	$10.6^{bcde} \pm 1.3$	$0.0^{a} \pm 0.0$
3.52	2x	$57.8^{b} \pm 5.0$	$60.8^{bc} \pm 5.9$	$75.5^{a} \pm 4.0$	$14.4^{b} \pm 2.0$	$0.0^{a} \pm 0.0$
Glufosinate-ammonium						
0.44	0.5x	$10.3^{fg} \pm 1.1$	$46.2^{cdef} \pm 3.9$	$44.1^{cd} \pm 8.2$	$0.6^{de} \pm 0.3$	$0.0^{a} \pm 0.0$
0.88	1x	$40.2^{d} \pm 5.6$	$55.6^{bcd} \pm 5.8$	$53.3^{bcd} \pm 8.6$	$5.1^{bcde} \pm 0.6$	$0.0^{a} \pm 0.0$
1.76	2x	$58.3^{b} \pm 4.3$	$81.4^{a} \pm 8.2$	$59.3^{abc} \pm 6.9$	$33.7^{a} \pm 6.5$	$0.0^{a} \pm 0.0$
Metsulfuron-methyl						
0.015	0.5x	$42.2^{cd} \pm 5.0$	$36.7^{efg} \pm 6.4$	$35.2^{d} \pm 7.9$	$5.3^{bcde} \pm 8.5$	$0.0^{a} \pm 0.0$
0.03	1x	$54.0^{bc} \pm 7.0$	$53.7^{bcde} \pm 8.9$	$48.2^{cd} \pm 5.0$	$6.1^{bcde} \pm 1.3$	$0.0^{a} \pm 0.0$
0.06	2x	$71.1^{a} \pm 3.2$	$70.3^{ab} \pm 3.6$	$68.5^{ab} \pm 6.4$	$12.7^{bc} \pm 4.3$	$0.0^{a} \pm 0.0$
Control		$0.0^{g} \pm 0.0$	$0.0^{h} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.0^{a} \pm 0.0$

Values in the same column followed by superscript similar letter(s) are not significantly different by DMRT ($P<0.05$). Data are presented as mean values (standard error) of three replicates at each exposure period. RFR, Recommended field rate; the rate which is recommended in the product label to apply in the field.

colony development relative to the control are shown in Table 2. The herbicides caused higher inhibition to bacterial population development compared with that of the fungi. At all sampling times and treatment rates of the herbicides, the inhibition percentages of bacterial colonies were higher than those observed for the fungal colony development, except for the glufosinate-ammonium treatment at 4 DAT and 6 DAT.

The highest inhibitions of the bacterial population were from 77.9 to 87.9%. These highest inhibitions, however, were observed from the 2 times recommended field rate for all herbicides. Treatment of herbicides at 0.5, 1 and 2 times their recommended field rate also indicated increased inhibition percentages with the increased in the herbicide rates, when sampled at 2 days after treatment until 10 DAT. However, the lowest treatment at 0.5 times the field recommended rate had also caused significantly high inhibition of the colony development compared with the control, and comparable with those of treatments at recommended field rate.

At the recommended field rate, the herbicides could be considered as moderately to highly toxic to bacterial population. Highest inhibition of bacterial growth was recorded at 68.7, 74 and 82% at 4 DAT for metsulfuron-methyl, glyphosate and paraquat, respectively, and 73% for glufosinate-ammonium at 2 DAT. However, glufosinate-ammonium caused the maximum suppression through growth inhibition of the bacterial colony development (73%) at faster rate (2 DAT) than paraquat, glyphosate and metsulfuron-methyl with 45.5, 55 and 67.3%, respectively. The inhibition percentages of bacterial population for all treatments reduced significantly by 10 DAT with a range of 8 to 22.8%. The observations were comparable with that observed for the fungal colony development discussed earlier. No inhibition at 20 DAT indicates that the bacterial population recovers from the earlier effects, similar to the fungal population.

Paraquat, glyphosate, glufosinate-ammonium and metsulfuron-methyl treatment to soil also affected the development of actinomycetes population (Table 3). The growth inhibition of actinomycetes colonies caused by the herbicides was similar to those recorded for the fungi and bacteria, which increased with the increased of the herbicides application rates. However, treatments at 0.5x and 1x the recommended field rates were significantly

Table 2. Effect of herbicide treatments on soil bacterial population at five exposure periods in soil microcosms.

Herbicide Treatments (mg a.i./g)		% Population inhibition relative to control (Mean ± SE)				
	RFR	**2 DAT**	**4 DAT**	**6 DAT**	**10 DAT**	**20 DAT**
Paraquat						
0.44	0.5x	$30.1^{e} \pm 1.0$	$73.6^{bc} \pm 2.5$	$62.2^{b} \pm 5.2$	$17.8^{bc} \pm 3.1$	$0.0^{a} \pm 0.0$
0.88	1x	$45.5^{d} \pm 3.0$	$82.0^{ab} \pm 3.5$	$67.9^{b} \pm 4.9$	$22.8^{ab} \pm 6.5$	$0.0^{a} \pm 0.0$
1.76	2x	$46.7^{d} \pm 3.7$	$87.9^{a} \pm 4.5$	$82.9^{a} \pm 2.6$	$32.9^{a} \pm 1.4$	$0.0^{a} \pm 0.0$
Glyphosate						
0.88	0.5x	$52.8^{d} \pm 0.9$	$73.3^{bc} \pm 4.7$	$63.5^{b} \pm 2.2$	$13.3^{bcd} \pm 4.9$	$0.0^{a} \pm 0.0$
1.76	1x	$55.0^{cd} \pm 8.3$	$74.0^{bc} \pm 5.8$	$67.9^{b} \pm 4.4$	$14.6^{bc} \pm 2.7$	$0.0^{a} \pm 0.0$
3.52	2x	$67.0^{bc} \pm 0.8$	$81.0^{ab} \pm 2.6$	$83.7^{a} \pm 2.4$	$18.3^{bc} \pm 6.2$	$0.0^{a} \pm 0.0$
Glufosinate-ammonium						
0.44	0.5x	$69.5^{ab} \pm 6.1$	$39.0^{d} \pm 3.4$	$27.1^{c} \pm 6.1$	$0.6^{d} \pm 0.6$	$0.0^{a} \pm 0.0$
0.88	1x	$73.0^{ab} \pm 3.7$	$48.0^{d} \pm 3.7$	$30.9^{c} \pm 2.3$	$8.0^{cd} \pm 5.3$	$0.0^{a} \pm 0.0$
1.76	2x	$82.1^{a} \pm 2.3$	$66.6^{c} \pm 4.4$	$35.7^{c} \pm 1.1$	$17.0^{bc} \pm 2.1$	$0.0^{a} \pm 0.0$
Metsulfuron-methyl						
0.015	0.5x	$53.8^{d} \pm 5.3$	$68.1^{c} \pm 3.5$	$58.9^{b} \pm 2.3$	$10.4^{bcd} \pm 6.2$	$0.0^{a} \pm 0.0$
0.03	1x	$67.3^{bc} \pm 3.9$	$68.7^{c} \pm 4.8$	$59.7^{b} \pm 1.0$	$17.3^{bc} \pm 5.0$	$0.0^{a} \pm 0.0$
0.06	2x	$69.6^{ab} \pm 5.5$	$77.9^{abc} \pm 3.2$	$67.2^{b} \pm 7.4$	$21.1^{abc} \pm 1.8$	$0.0^{a} \pm 0.0$
Control		$0.0^{f} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.0^{d} \pm 0.0$	$0.0^{d} \pm 0.0$	$0.0^{a} \pm 0.0$

Values in the same column followed by superscript similar letter(s) are not significantly different by DMRT ($P<0.05$). Data are presented as mean values (standard error) of three replicates at each exposure period. RFR, Recommended field rate; the rate which is recommended in the product label to apply in the field.

lower than that at 2x the recommended field rate.

Herbicides, at rates recommended for use in the field, were considered as moderately toxic to actinomycetes population in soil. Highest inhibition at the recommended field rate for all herbicides were 70.6, 47.0, 64.3 and 59.4% for paraquat, glyphosate, glufosinate-ammonium and metsulfuron-methyl, respectively. These inhibition percentages were observed by 4 DAT for paraquat, glyphosate and metsulfuron-methyl, whereas it was slower for glufosinate-ammonium, observed at 6 DAT. By 10 DAT, however, the inhibition rate for actinomycetes were still relatively high, in comparison with that of the earlier sampling period, and also to that of the fungal and bacterial populations. This could indicate slower recovery period of actinomycetes after the initial effect of the herbicides. However, by 20 DAT, no further inhibition to the actinomycetes population was observed for all treatments, which indicate full recovery from the treatment.

DISCUSSION

Herbicide treatments of paraquat, glyphosate, glufosinate-ammonium and metsulfuron-methyl showed significant effects on microbial growth and development in soil environment. Significant increased of fungal, bacterial and actinomycetes growth inhibition were observed from 0.5x to 2x their recommended field application rates, indicating a positive correlation between growth inhibition and treatment rates. Bacterial and actinomycetes populations were severely affected by Paraquat which inhibited their population growth by 70 to 82% at recommended field rate. However, the fungal population in soil was moderately inhibited (54.3%). Paraquat has also been reported to inhibit several microorganisms in soil by Smith and Mayfield (1977). They reported that paraquat could inhibit a great number of cellulolytic microflora and that might cause injurious effects to symbiotic, anaerobic and nitrogen fixing microorganisms. Paraquat is also known to be bounded strongly and coherently to soil components, including clay minerals and organic matter, therefore limits the access of microorganisms to paraquat in soil water (Bromilow, 2003; Isenring, 2006). Thus, adsorption of paraquat to soil rapidly decreases the bioavailability of the herbicide in the soil environment and demonstrated the capability of adsorption process to deactivate hundreds or even thousands of paraquat application over many soil types (Roberts et al., 2002). The sandy clay classification of the

Table 3. Effect of herbicide treatments on soil actinomycete population at five exposure periods in soil microcosms.

Herbicide Treatments (mg a.i./g)		% Population inhibition relative to control (Mean ± SE)				
	RFR	**2 DAT**	**4 DAT**	**6 DAT**	**10 DAT**	**20 DAT**
Paraquat						
0.44	0.5x	$29.7^{cd} \pm 7.4$	$68.7^{ab} \pm 5.8$	$36.5^{d} \pm 6.5$	$26.3^{bc} \pm 3.1$	$0.0^{a} \pm 0.0$
0.88	1x	$31.1^{cd} \pm 8.5$	$70.6^{ab} \pm 7.2$	$45.1^{cd} \pm 4.4$	$31.4^{bc} \pm 2.5$	$0.0^{a} \pm 0.0$
1.76	2x	$40.6^{bc} \pm 4.3$	$82.5^{a} \pm 0.8$	$60.6^{b} \pm 8.9$	$47.4^{a} \pm 3.3$	$0.0^{a} \pm 0.0$
Glyphosate						
0.88	0.5x	$11.9^{ef} \pm 6.0$	$38.2^{d} \pm 6.7$	$20.1^{e} \pm 3.0$	$11.2^{de} \pm 5.1$	$0.0^{a} \pm 0.0$
1.76	1x	$19.4^{de} \pm 7.2$	$47.0^{cd} \pm 3.4$	$22.7^{e} \pm 3.8$	$20.9^{cd} \pm 8.4$	$0.0^{a} \pm 0.0$
3.52	2x	$28.5^{cde} \pm 1.8$	$55.8^{bc} \pm 7.6$	$38.4^{d} \pm 4.1$	$25.6^{bc} \pm 7.9$	$0.0^{a} \pm 0.0$
Glufosinate-ammonium						
0.44	0.5x	$0.0^{f} \pm 0.0$	$0.0^{e} \pm 0.0$	$60.9^{b} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.0^{a} \pm 0.0$
0.88	1x	$0.0^{f} \pm 0.0$	$10.4^{e} \pm 2.0$	$64.3^{b} \pm 0.7$	$0.0^{e} \pm 0.0$	$0.0^{a} \pm 0.0$
1.76	2x	$11.9^{ef} \pm 6.0$	$46.5^{cd} \pm 6.8$	$79.2^{a} \pm 0.0$	$26.5^{bc} \pm 5.0$	$0.0^{a} \pm 0.0$
Metsulfuron-methyl						
0.015	0.5x	$23.9^{cde} \pm 5.6$	$57.0^{bc} \pm 6.6$	$54.2^{bc} \pm 3.5$	$26.9^{bc} \pm 4.0$	$0.0^{a} \pm 0.0$
0.03	1x	$48.2^{b} \pm 4.6$	$59.4^{bc} \pm 6.3$	$57.5^{bc} \pm 6.4$	$38.8^{ab} \pm 1.7$	$0.0^{a} \pm 0.0$
0.06	2x	$69.1^{a} \pm 7.0$	$79.9^{a} \pm 1.8$	$63.3^{b} \pm 3.4$	$51.3^{a} \pm 4.8$	$0.0^{a} \pm 0.0$
Control		$0.0^{f} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.0^{f} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.0^{a} \pm 0.0$

Values in the same column followed by superscript similar letter(s) are not significantly different by DMRT ($P<0.05$). Data are presented as mean values (standard error) of three replicates at each exposure period. RFR, Recommended field rate; the rate which is recommended in the product label to apply in the field.

experimental soils might have reduced the binding of paraquat to soil components and thus increasing the availability of paraquat in soil water, and hence affecting the soil microorganisms significantly.

Glyphosate was observed to be less toxic than paraquat to bacterial and actinomycetes populations. At recommended field rate, it inhibited the bacterial population by 74%. The inhibition of actinomycetes and fungal populations were moderate with 47 to 59.3%. Findings from this study were supported by other studies (Anderson and Kolmer, 2005; Franz et al., 1997; Mekwatanakarn and Sivasithamparam, 1987; Toubia-Rahme et al., 1995; Turkington et al., 2001; Wong et al., 1993; Wyss and Muller-Scharer, 2001). However, few studies contradict this result (Busse et al., 2001; Muller et al., 1981; Stratton and Stewart, 1992; Wardle and Parkinson, 1990; Weaver et al., 2007). As a weed killer, glyphosate targets a single enzyme called 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Franz et al., 1997) which plays important role in the shikimic acid pathway responsible for biosynthesis of aromatic amino acids, and this enzyme is widely present in plants and microorganisms, including bacteria and fungi (Kishore and Shah, 1998; CaJacob et al., 2004). The presence of EPSPS proteins in bacteria and fungi, therefore, made the microorganisms vulnerable to glyphosate. CaJacob et al. (2004) also reported that EPSPS proteins have been isolated and characterized from microorganisms, which some can tolerate glyphosate while others were sensitive to the herbicide.

Glufosinate-ammonium was considered to be more toxic than glyphosate to the actinomycetes population. The inhibition of bacterial population by glufosinate-ammonium (73%) was considered as being equally toxic compared with glyphosate (74%). The growth-inhibition by glufosinate-ammonium could be due to negative effects on the dehydrogenase activity of soil microorganisms as explained by Pampulha et al. (2007), and subsequent decline of growth-inhibition likely due to the compound's rapid degradation process in soil (Ismail and Ahmed, 1994). A study done by Ahmad and Malloch (1995) reported that bacterial growth was reduced only about 40% by glufosinate-ammonium herbicide in agricultural soils. Similarly, the herbicide at recommended field rate reduced the bacterial population temporarily, as they recovered after 7 days (Ismail et al.,

1995). Pampulha et al. (2007) reported significant inhibition in growth of actinomycetes, *Streptomyces* spp., within six days after application of the herbicide to soil microcosms. In contrary, Ahmad and Malloch (1995) obtained insignificant result for the effects of glufosinate-ammonium towards soil actinomycetes.

Metsulfuron-methyl was observed to be the least toxic to fungal and bacterial populations compared to paraquat, glyphosate and glufosinate-ammonium. However, the toxicity of metsulfuron-methyl to actinomycetes population was higher than glyphosate, but similar to paraquat and glufosinate-ammonium. Metsulfuron-methyl could be considered as being moderately toxic to bacterial, actinomycetes and fungal populations at recommended field rate. Ismail et al. (1996) showed that bacterial population decreased when the concentrations of metsulfuron-methyl increased during the first 3 to 9 days after application, depending on soil types. However, Ismail et al. (1996) also demonstrated increase in fungal population with increasing metsulfuron-methyl concentrations, which may be influenced by the soil type. El-Ghamry et al. (2000) reflected the toxicity effect of metsulfuron-methyl when the soil microbial biomass significantly decreased with increasing concentrations of the herbicide, which could either be due to toxicity effect and the adsorption of the herbicide in soil or because the soil microorganisms were not adapted to the herbicide itself.

In this study, the herbicide treatments to soil indicated short term growth-inhibitory effects on soil microbial population. The treatment effects on soil microbial population growth over the five exposure periods exhibited rapid decreasing trends after 6 DAT, and the effects were zero at 20 DAT which indicate full recovery of the microbial populations from the initial herbicidal effects. This was to be expected because the amounts of herbicides molecules present in the soil were negligible to have any influence on fungal population that ultimately lead to zero inhibition of fungal growth.

CONCLUSION

Paraquat, glyphosate, glufosinate-ammonium and metsulfuron-methyl caused significant inhibitory effects on growth of fungal, bacterial and actinomycetes populations in soil microcosms. However, the exposures of the microorganisms upon herbicide applications cause short term changes on the growth and development of the microbial community in oil palm plantation soil.

ACKNOWLEDGEMENTS

Sincere thank is due to Universiti Putra Malaysia (UPM) for funding this study under the Research University Grant Scheme (RUGS).

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