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Growth-inhibitory Effects of Herbicides on Soil Bacterial Population in Oil Palm Plantation

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Herbicides are commonly used for controlling weeds in oil palm plantations, but their residues in soil were reported to affect microorganism development which influences many crucial biological processes in soil. The study was conducted to assess the growth-inhibitory effects on bacterial populations exposed to 0.5, 1 and 2 times recommended field application rates of four herbicides (paraquat, glyphosate, glufosinate-ammonium and metsulfuron-methyl) in pure culture media (*in vitro*) and in incubated soil microcosm. Herbicides treated to culture media caused significant reduction to growth of bacterial species (*Bacillus humi, B. cereus/thuringiensis, B. pseudomycoides*, *Bacillus* sp.). Among the species, *B. humi* was the most sensitive species to all the herbicides, whereas *M. spinosa* was comparatively least affected, except to paraquat. In soil microcosm, the inhibitory percentages at recommended field application rates were highest for paraqaut (82.0%) followed by glyphosate (74.0%), glufosinate-ammonium (73.0%) and metsulfuron methyl (68.7%). However, the inhibition percentages over five exposure periods (2, 4, 6, 10, 20 DAT, days after treatment) were observed to be of higher levels from the initial effect until 6 DAT, and decreased rapidly by 10 DAT. No inhibition was observed by 20 DAT. Assessment of the exposure data presented here indicates that the above-mentioned herbicides used in oil palm plantation would have the potential of being toxic to the bacterial populations upon direct exposure *in-vitro*. However, natural processes in soil lower the inhibitory effect on the bacterial growth, which was transient, and that the bacterial populations were able to recover fully from the initial shock.

Key words: Exposure period, Herbicide toxicity, *in vitro*, Recommended field rate, Soil microcosm, Soil microorganisms.

Wide-scale use of herbicides in agriculture is common in augmenting its yield, but the ultimate 'sink' of the applied herbicides is the soil. Soil, however, is the storehouse of multitude of microbes, in quantity and quality, which play important roles in soil ecosystem where they carry out crucial role in nutrient cycling and decomposition[1]. The major groups of microorganism that make up the soil microbial population include bacteria, actinomycetes and fungi[2] those carry out almost all known biological reactions in soil[3]. Fertile soils contain higher bacterial number, and studies on estimation of soil fertility by microbial numbers found that most pathways into the nutrient pool were facilitated by soil bacteria[4].

Herbicides reaching the soil in significant quantities could have direct effect on soil microbial development[5,6] Longer persistence of herbicides leading to accumulation of residues in soil may also result in increased toxic effects on soil microorganisms and their activities, which in turn influence the soil fertility[7]. An ideal herbicide

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should have the quick ability to be degraded into non-toxic substances that ultimately exert less toxic effects on soil microbes[8]. The response of soil microbial activity to herbicide treatments showed significant variability among herbicides[9]. Some species respond sensitively to particular herbicides resulting in reduced microbial number and activity shortly after herbicide application, depending on the application rate[10]. Reviews on the effect of herbicides on soil microorganisms have shown that herbicide chemicals when applied at excessive rates can inhibit or suppress the activities of soil microorganism[11]. However, herbicides are considered to have no major or long-term effect on the total bacterial populations in soil when applied at normal field recommended rates[12]. Thus, the herbicide effects on bacterial growth, either stimulating or depressive, depend upon the kind of chemicals, their concentrations, microbial species in soil, and possible interactions and moderations by environmental conditions[13, 6].

Several herbicides such as paraquat, glyphosate, glufosinte-ammonium and metsulfuron methyl are widely used in oil palm plantations in Malaysia to control weeds[14 -16]. According to Pampulha & Oliveira[17], excessive use of these chemicals has aroused attention about their effect to the environment, such as persistence in soil, the toxicity to soil microbes and also selection of resistant species. The non-target effects of these compounds become harmful to soil microorganisms that ultimately may reduce the performance of important soil functions[18]. Therefore, the study was designed to investigate the inhibitory effects of four types of herbicide on bacterial growth and population associated with oil palm plantation soil by direct exposure (*in vitro*) and in soil (soil microcosm).

MATERIALS AND METHODS

Herbicide treatments

Four different herbicide treatments consisted of paraquat (Gramaxone®, Syngenta Corporation Sdn. Bhd.), glyphosate (Roundup®, Monsanto), glufosinate-ammonium (Basta®15, Bayer Crop Sciences) and metsulfuron methyl (Ally® 20DF, DuPont Agrochemicals) were used in the study, and at three different concentrations (rates) of each: paraquat and glufosinate-ammonium at 0.44, 0.88 and 1.76 mg a.i./mL; glyphosate at 0.88, 1.76 and 3.52 mg a.i./mL; metsulfuron methyl at 0.015, 0.03 and 0.06 mg a.i./mL. These treatment rates represented 0.5, 1 and 2 times (x) their recommended field application 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:

$$\text{X mL/L media} \quad \frac{\text{Desired field rate (g a.i./ha) x 1000 mL}}{\text{Amount of a.i. in formulation (g a.i./L) x 450 L/ha x 1 L}}$$

In vitro experiment

A total of five bacterial species (*Bacillus. humi*, *B. cereus/thuringiensis*, *B. pseudomycoides*, *Bacillus* sp. and *Malikia spinosa*) which were isolated and identified (using Biolog Gen II Technology) from oil palm plantation soils at UPM (Universiti Putra Malaysia), were studied for their sensitivity to different concentrations of herbicide treatments. Serial dilutions from the stock culture of each species were made aseptically under the laminar flow by adding 1 mL of the bacterial suspension with OD (optical density) reading range of 0.7 to 0.8 (wavelength 620 nm) into a test tube containing 9 mL of sterile distilled water to make 10^{-1} dilution. The test tube was vortexed for approximately 10 sec to thoroughly mix the dilution. The dilution process was repeated until reaching dilution up to 10^{-6}, before pipetting on the herbicide-NA (nutrient agar, Oxoid) medium and control plates accordingly. The herbicide-NA media was prepared by adding each herbicide treatment into sterilized (121^0C, 15 min) NA (nutrient agar) media accordingly, and mixed thoroughly on the hotplate and stirrer (Jerway, Bibby Scientific Ltd., UK), before pouring into the petri dishes marked with lines dividing into 3 sections at the bottom. The NA medium without herbicide treatments served as control.

The test tube containing suspension of each bacterial species was vortexed before fives drops (10 µL drop^{-1}) of the diluted bacterial suspension (10^{-4} to 10^{-6}) were pipetted out. The drop plate technique was used where the pipetted suspension was placed onto each particular section of the herbicide-NA medium and control plates (marked by dividing lines) according to dilution value of the suspensions. The plates were covered and allowed to dry. After one hour, the plates were inverted, sealed with parafilm to avoid

contamination and incubated at 25 ^{0}C in dark incubator for 24 h.

Enumeration of bacterial population was evaluated by their colony development. The bacterial colony was examined and enumerated using Colony Counter (Rocker). The step was repeated daily for 3 consecutive days. The colony counts were averaged, and Colony-forming unit (CFU)/mL was calculated using the formula:

$$\text{CFU/mL} = \frac{\text{Colony-forming unit} \times \text{dilution factor}}{\text{Amount of aliquot}}$$

Soil microcosm experiment

Preparation of soil microcosm

Soils were collected from oil palm plantation at Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. Eighty soil cores (approximately 40 kg) were sampled to a depth of 15 cm using an auger, collected randomly from underneath the surrounding palms and between the palm rows. Several characteristics of the soils sampled from oil palm plantation were determined as shown in Table I.

The samples were mixed thoroughly to form a composite sample, which processed accordingly for microcosm treatments. The soil microcosms were prepared according to Pampulha & Oliveira[17] with minor modifications. The soils were air-dried slowly in the 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 moisture holding capacity (MHC). The moisture content was measured by the difference of weight between wet and dry (oven drying at 70 °C for 24 h) of specific amount of soil, calculated using the formula:

$$\text{Moisture content} = \frac{(\text{Weight of wet soil} - \text{Weight of dry soil})}{\text{Weight of wet soil}} \times 100$$

Whereas, moisture holding capacity (MHC) was determined by the difference in weight between saturated and drained-off soil, calculated using the formula:

$$\text{Moisture Holding Capacity (MHC)} = \frac{\text{Mass of water contained in saturated soil}}{\text{Mass of saturated soil}} \times 100$$

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 65 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 25ºC incubator, for 10 days to allow time for adaptation of microorganisms before treatment with the herbicides. The herbicides were applied aseptically under laminar flow unless stated otherwise. 50 ml of the herbicide solutions were sprayed to the soil in the glass bottles accordingly, using hand sprayer. The herbicide was mixed thoroughly by constant shaking for 5 min. Control soils were 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^{0}C. Sterile distilled water was added on weekly basis to restore the initial weight of each microcosm, maintaining the constant moisture content.

Sub-culture and enumeration of bacteria from soil microcosm

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 Co. Ltd., Korea) for 30 seconds to thoroughly mix them. This process was repeated until the dilutions were made up to 10^{-5} to complete the serial dilutions, before pipetting on the growth media. The growth media was Nutrient agar (NA, Oxoid) amended with 0.1 g/L cyclohexamide (Merck) prepared for growing bacteria. The inhibitor (cyclohexamide) was added into sterilized (121^{0}C, 15 min) media, and mixed thoroughly on hotplate and stirrer (Jenway, Bibby Scientific Ltd., UK), before pouring into each petridish, with marker line division at the bottom, dividing it into three sections.

The test tubes of the serial dilutions were vortexed before five drops (10 µL drop^{-1}) of bacterial suspension (10^{-3} to 10^{-5}) were pipetted out by drop

plate method onto each particular section of the media (marked by dividing lines) according to dilution value of the suspensions. The plates were prepared in triplicates, covered and allowed to dry for 1 h. The plates were inverted, sealed with parafilm to avoid contamination and incubated in darkness at 25ºC. After 24 h, the plates were examined for bacterial growth followed by enumeration of colonies using the Colony Counter (Rocker). The total up of the colonies was used to calculate the CFU/g dry weight of soil. Dry weight of soil was determined after oven drying at 70ºC for 24 h using the formula:

weight of moist soil x (1-%soil sample/100)

and the Colony-forming unit (CFU) was calculated using the formula:

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

Statistical analysis

The experiments were conducted using complete randomized design (CRD) with five replicates. Data on *in vitro* were analyzed following 2-way analysis of variance (ANOVA) between herbicides and each fungal species. However, data regarding soil microcosm were analyzed following 2-way ANOVA between herbicides and each exposure date. Mean separations were done by Duncan's multiple range test (DMRT) using statistical analysis system (SAS)[19]. Results were expressed as a percentage of fungal growth inhibition. Significant differences were accepted at $p < 0.05$.

RESULTS

Effects of herbicides in vitro on population of bacterial species

The effect of herbicide treatments on bacterial species was determined based on the inhibition percentages of the growth of bacterial colonies in each treatment media. The herbicides inhibited the growth of the bacterial colonies significantly at their respective rates compared with the control (Table 2). The inhibition increased with increased herbicide concentrations, and bacterial species showed different degree of sensitivity to the herbicide compounds. Treatments at 0.5, 1 and 2x recommended field application rate of paraquat completely inhibited (100%) the colony growth of all five bacterial species. However, only growth of *B. humi, B. cereus/thuringiensis, B. pseudomycoides* and *Bacillus* sp. showed 100% inhibition on glyphosate treated media. Whereas, *M. spinosa* showed high degree of tolerance to glyphosate *in vitro*. At glyphosate concentration of 0.5, 1 and 2x recommended field application rates, *M. spinosa* showed 1.5%, 8.8% and 14.6%, growth inhibition, respectively. Glufosinate-ammonium treatments produced similar trend of inhibition responses on the bacterial population to glyphosate, causing 100% inhibition of growth to four bacterial species, but *M. spinosa* showed 6.9%, 15.7% and 29.9% inhibition percentages at 0.5. 1 and 2x recommended field application rates, respectively. The inhibition percentages on *M. spinosa*, however, were comparatively higher than those for glyphosate.

Inhibition of growth of the bacterial species was substantially lower in treatments with metsulfuron methyl in comparison with those treated with paraquat, glyphosate and glufosinate-ammonium. Growth of *B. humi* was the most inhibited species attaining 17.2 %, 74.7% and 100% for treatments at 0.5, 1 and 2 times recommended field application rate, respectively. *B. pseudomycoides* was observed to be the least sensitive to metsulfuron methyl treatments *in vitro* even at 2x recommended field application rate. Growth of the bacterial species *M. spinosa, B. cereus/thuringiensis and Bacillus* sp. were also least inhibited by the metsulfuron methyl treatments, with maximum inhibitions for the three

Table 1. Characteristics of the soil sampled from oil palm plantation, UPM

Soil texture	Soil pH	Soil chemical properties					
		% C	Total N (%)	Total P (ppm)	Available K (ppm)	Ca (ppm)	Mg (ppm)
Sandy clay(40% clay, 10% silt, and 50% sand)	4.1 ± 0.01	1.94	0.32	219	104	119	32

populations at recommended field application rate were 15.7 %, 10.8% and 3%, respectively.

Effect of herbicides on bacterial population in soil microcosms

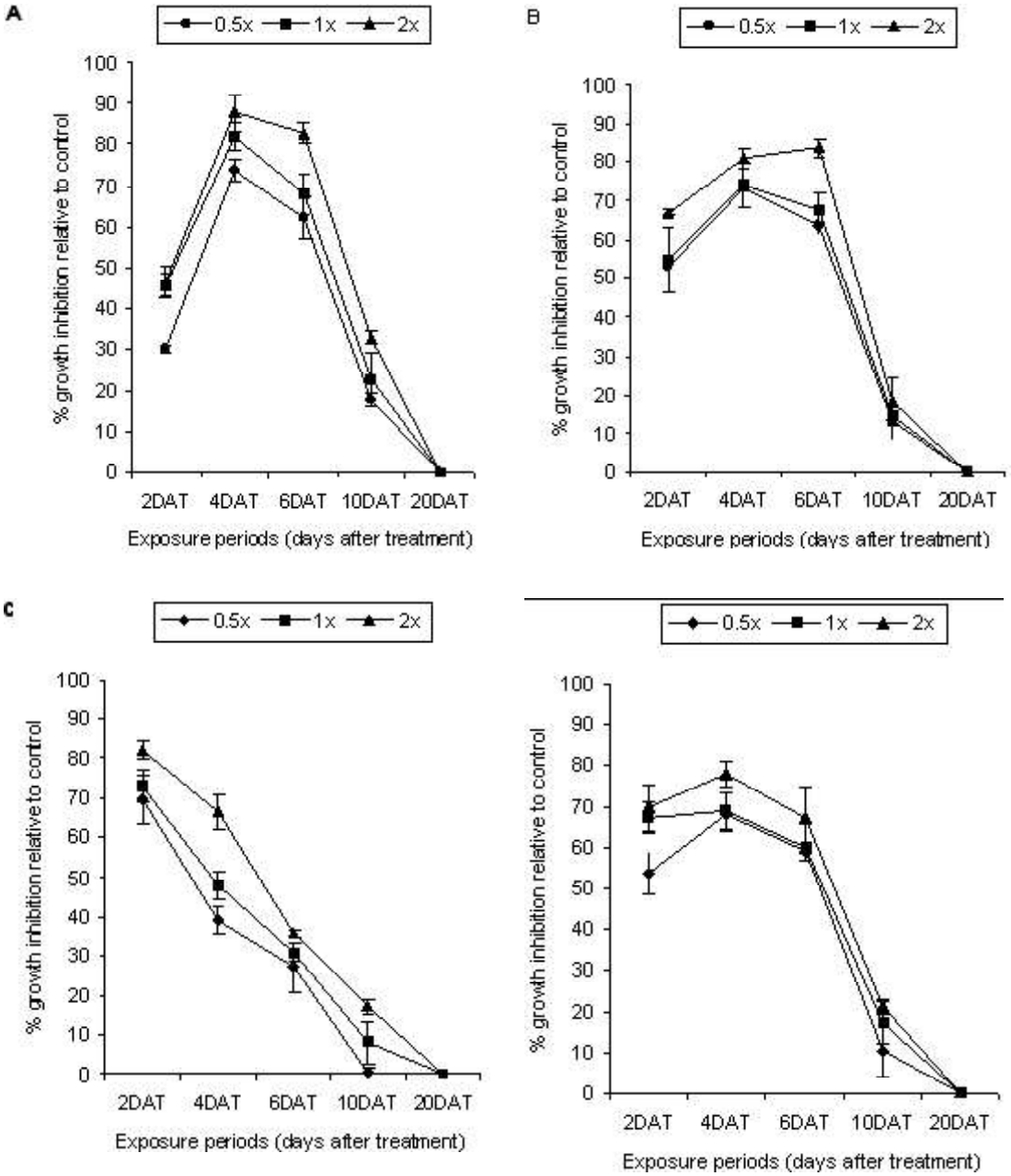
The effect of herbicides on bacterial population in soil microcosms was evaluated by their colony development. Bacterial populations in soil were affected significantly by the application of paraquat, glyphosate, glufosinate-ammonium and metsulfuron methyl herbicides. Observation indicated that the inhibition percentages of the colony development increased with increased application rates of each herbicide at 0.5, 1 and 2x their recommended field rate (Fig. 1). The inhibition percentages of bacterial colony were varied significantly at sampling dates between 2 DAT and 10 DAT. The highest inhibitions of the bacterial population were observed at 2x recommended field application rates for all herbicides from initial 2 DAT until 6 DAT, which ranged from 35.7% to 87.9%. However, during the same exposure period, herbicide treatments at the recommended field application rate (1x) also caused significant inhibition to bacterial population which ranged from 30.9 to 82.0%.

Increasing trend of inhibition on growth of bacterial populations was observed from the initial effect until 4 DAT for all herbicides, except to glufosinate-ammonium which was gradually declined onward from the initial effect at 2 DAT. Inhibition percentages of bacterial growth for paraquat, glyphosate and metsulfuron methyl was highest at 4 DAT with 82%, 74% and 68.7%, respectively, whereas, the highest inhibition percentage (73%) for glufosinate-ammonium was obtained at 2 DAT. The inhibition percentages of bacterial population for all treatments were reduced significantly by 10 DAT with a range of 8% to 22.8%. However, no inhibition of bacterial population for all herbicides was observed at 20 DAT.

Table 2. Percent growth inhibition of five bacterial species *in vitro* (nutrient agar) to three different concentrations for each of the four herbicides compared with control. Data are presented as mean values (standard error) of five replicates at each herbicide concentration

Herbicide treatment		% Growth inhibition of bacterial species relative to control(Mean ± SE[3])				
Concentration[2] (rates)	RFR[1]	*B. humi*	*B. cereus/ thuringiensis*	*B. pseudomycoids*	*Bacillus sp.*	*M. spinosa*
Paraquat						
0.44 mg a.i./mL	0.5x	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$
0.88 mg a.i./mL	1x	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$
1.76 mg a.i./mL	2x	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$
Glyphosate						
0.88 mg a.i./mL	0.5x	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$1.5^{e} \pm 1.5$
1.76 mg a.i./mL	1x	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$8.8^{cde} \pm 4.4$
3.52 mg a.i./mL	2x	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$14.6^{cd} \pm 5.0$
Glufosinate-ammonium						
0.44 mg a.i./mL	0.5x	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$6.9^{de} \pm 3.4$
0.88 mg a.i./mL	1x	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$15.7^{cd} \pm 4.8$
1.76 mg a.i./mL	2x	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$29.9^{b} \pm 3.1$
Metsulfuron methyl						
0.015 mg a.i./mL	0.5x	$17.1^{c} \pm 4.2$	$0.6^{c} \pm 0.6$	$0.0^{c} \pm 0.0$	$1.5^{c} \pm 0.9$	$0.0^{e} \pm 0.0$
0.03 mg a.i./mL	1x	$74.7^{b} \pm 2.8$	$10.8^{b} \pm 4.8$	$0.0^{c} \pm 0.0$	$3.0^{c} \pm 1.2$	$15.7^{cd} \pm 5.2$
0.06 mg a.i./mL	2x	$100.0^{a} \pm 0.0$	$13.9^{b} \pm 4.1$	$2.1^{b} \pm 1.2$	$40.8^{b} \pm 10.6$	$18.4^{c} \pm 4.6$
Control		$0.0^{d} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$0.0^{e} \pm 0.0$

[1]RFR, Recommended field rate; the rate which is recommended in the product label to apply in the field
[2]Concentrations of herbicide are calculated at 0.5x, 1x and 2x of the recommended field rate.
[3] SE, Standard Error
Mean values in the same column followed by similar superscript letter(s) are not significantly different by DMRT ($P < 0.05$).



DAT, days after treatment
Bars depict standard error (SE) of mean.
0.5x (line with solid diamond), 1x (line with solid square) and 2x (line with solid triangle) represent the three herbicide concentrations in terms of the recommended field rate

Fig. 1. Percent growth inhibition of bacterial population in soil microcosm to three different concentrations for each of the four herbicides under five exposure periods compared with control - (A) paraquat, (B) glyphosate, (C) glufosinate-ammonium, and (D) metsulfuron methyl. Data are presented as mean values (standard error) of five replicates at each exposure period

DISCUSSION

Effects of herbicides in vitro on population of bacterial species

Paraquat, glyphosate, glufosinate-ammonium and metsulfuron methyl at 0.5, 1 and 2x their recommended field application rates significantly inhibited the growth of bacterial species. However, the growth inhibition levels for most of the species by paraquat, glyphosate and glufosinate-ammonium were higher, whereas, metsulfuron methyl showed lower inhibitions.

Paraquat was highly toxic to bacterial (*B. humi, B. cereus/thuringiensis, B. pseudomycoides*, *Bacillus* sp. and *M. spinosa*) populations *in vitro* causing total inhibitions (100%) to the colony development observed at all treatment concentrations. Similar response was also observed by Leach *et al*.[20] on the effect of several herbicides, including paraquat at 1, 10 and 100 ppm amended in trypticase soy and simmons citrate agar media to soil-borne bacteria which included *Azotobacter chroococcum, Bacillus subtilis, Erwinia*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*.

Glyphosate and glufosinate-ammonium were also highly toxic *in vitro*, causing 100% inhibition of growth of four bacterial species (*B. humi, B. cereus/thuringiensis, B. pseudomycoides* and *Bacillus* sp.), whereas, *M. spinosa* was least affected by this two herbicides. Inhibition of bacterial species by glyphosate was explained to enzymetic inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the microorganisms[21,22]. Busse *et al*.[23] explained that growth of all four *Bacillus* spp. (*B. humi, B. cereus/ thuringiensis, B. pseudomycoides* and *Bacillus* sp.) were totally inhibited by glyphosate with 100% inhibition as because glyphosate was lethal to bacteria when added to soil-free media due to the absence of glyphosate-adsorptive material. Less inhibition of glyphosate and glufosinate-ammonium to *M. spinosa* could be related to the gram reactions of bacterial species, in which *M. spinosa* was the only gram negative species in this study. Moneke *et al*.[24] found that some gram negative bacteria such as *Pseudomonas fluorescens* and *Acetobacter* sp. have the ability to degrade glyphosate under varying environmental conditions. However, *M. spinosa* could also be tolerant to glufosinate-ammonium due to the gram negative properties of the species. A list of gram negative bacterial strains which includes *Burkholderia sacchari, Serratia marcescens* and *Pseudomonas psychrotolerans* were also reported to be tolerant to glufosinate-ammonium herbicide[25], whereas, a gram positive bacteria such as *Bacillus pumilus* had been classified as glufosinate-sensitive soil bacterium[26].

Metsulfuron methyl could be considered as being the least toxic to bacterial (*B. humi, B. cereus/thuringiensis, B. pseudomycoides*, *Bacillus* sp. and *M. spinosa*) species *in vitro* compared to paraquat, glyphosate and glufosinate-ammonium herbicides. The low toxicity of metsulfuron methyl to the bacterial species could indicate their positive role in the soil biological processes such as degradation. At recommended field application rate of metsulfuron methyl, the growth inhibitions were observed to be significantly low (0-15.7% range) for bacterial species, except to *B. humi* (74.7%). Metsulfuron methyl, therefore, can be considered to be highly toxic to *B. humi* with inhibition of growth reaching 100% at treatment of 2x recommended field application rate. According to Boldt & Jacobsen[27], the growth-reducing effects of metsulfuron methyl on bacterial isolates were caused by an inhibition of the enzyme acetolactate synthase in the organisms.

Effect of herbicides on bacterial population in soil microcosms

The effects of paraqaut, glyphosate, glufosinate-ammonium and metsulfuron methyl sprayed to soil at 0.5, 1 and 2 times their recommended rates showed variation among different exposure periods. Significant inhibitions of the growth of bacterial populations, however, were within 1 week exposure period after the treatment. The inhibition percentages were lower when herbicide treatments were applied to soil microcosms, compared with the treatments *in vitro.* This is because of the immediate and direct exposure of the bacterial populations to herbicides in the pure *in vitro* culture media. Similar findings were reported by Busse *et al*.[23].

In the soil microcosm, all herbicides indicated moderate to high toxicity to the bacterial populations at recommended field rate. The bacterial response upon herbicide exposure demonstrated that paraquat was able to inhibit

bacterial population in soil. Paraquat observed to be the most toxic to bacterial populations that exhibited the peak inhibition at 4 DAT, and followed by a gradual decline in the inhibition percentages through adsorption to soil that decreases the bioavailability of the herbicide in the soil environment[28]. However, the sandy clay classification of the experimental soils might have reduced the binding of paraquat to soil components, increasing the availability of paraquat in soil water, thus affecting the soil microorganisms significantly at initial exposure periods. Similar to the findings by Smith & Mayfield[29] on paraquat that inhibited a great number of cellulolytic microflora, and might cause early injurious effects to symbiotic, anaerobic and nitrogen fixing microorganisms in soil.

On the other hand, the inhibition caused by glyphosate and glufosinate-ammonium was considered as toxic to the bacterial populations. Significant inhibitions by glyphosate were until 6 DAT with highest at 4 DAT. However, the inhibition of bacterial population by glufosinate-ammonium was highest at first 2 DAT, after which it gradually declined onward until 6 DAT, and by 10 DAT very lower inhibitory effect on bacterial population was observed. Ismail *et al*.[30] found that the herbicide at recommended field rate reduced the bacterial population temporarily, as they recovered after 7 days. Significant inhibition of bacterial species by glyphosate in soil were reported by Anderson & Kolmer[31]; Franz *et al*.,[21]. In addition, glufosinate-ammonium was reported to reduce 40% of bacterial growth[32], where the herbicide was rapidly inactivated and biologically transformed to degradation products[33].

Metsulfuron methyl was observed to be the least toxic to the bacterial population in soil compared with paraquat, glyphosate and glufosinate-ammonium. Metsulfuron methyl could be considered as being moderately toxic to bacterial populations at recommended field rate. El-Ghamry *et al*.[34] 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. This was supported by reports on the removal of metsulfuron methyl through bacterial degrading activities in soil[35,36], and Ismail *et al*.[37] showed that bacterial population decreased when the concentrations of metsulfuron methyl increased during first 3-9 days after application, depending on soil types. Voets *et al*.[38] found that the degradation rate of sulfonylurea herbicides in the soil was positively correlated with the size of bacterial populations. This could indicate a significant potential of metsulfuron methyl to be of minimal toxicity to the bacterial populations.

CONCLUSION

In the present study, paraquat, glyphosate, glufosinate-ammonium and metsulfuron methyl at 0.5, 1 and 2 times their recommended field application rates showed inhibitory effects both *in vitro* and in soil microcosm treatments on bacterial populations obtained from soil of oil palm plantation. Increased inhibition of bacterial growth was found with increasing levels of treatment rates. The levels of growth-inhibiting effects of herbicides to bacterial species followed the rank order: Paraquat > glufosinte ammonium > glyphosate > metsulfuron methyl. The majority of the isolates (*B. humi, B. cereus/thuringiensis, B. pseudomycoides*, *Bacillus* sp.) tested *in vitro* were highly sensitive (higher percentage of inhibition) to paraquat, glyphosate and glufosinate-ammonium, but least sensitive to metsulfuron methyl. Among the bacterial species, *M. spinosa* was comparatively less sensitive to all herbicides except paraquat. In soil microcosm, the major inhibitory effect of the herbicides on the bacterial population growth and development was observed from 2 DAT until 6 DAT, and significantly decreased by 10 DAT. By 20 DAT, no inhibition on bacterial population growth was observed, which indicate recovery of the microbial populations from the initial herbicidal effects. However, future integrative research is essential to study more precisely the role of microbes on herbicide degradation and how these herbicides affect on non-degrading members of the microbial community in soil environment.

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