INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
08–309/AKA/2009/11–2–214–216
http://www.fspublishers.org

Full Length Article

Residual Phytotoxicity Effects of Paraquat, Glyphosate and Glufosinate-Ammonium Herbicides in Soils from Field-Treated Plots

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ABSTRACT

Soil residual phytotoxicity of commonly used herbicides in plantation crops in Malaysia were investigated through bioassay. Paraquat (Gramoxone^R) and glufosinate-ammonium (Basta^R) at 200, 400, 600 and 800 g a.i. ha^{-1}, and glyphosate (Round-up^R) at 400, 800, 1200 and 1600 g a.i. ha^{-1} were applied to field plots of 5 x 20 m^2. Cucumber and corn were used in the bioassay to test the residual effect of herbicides in the soil of the treated plots. Soil, sampled at 1 day after treatment (DAT) and until 2 weeks (WAT) later, did not affect the seed germination and seedling development of cucumber and corn. Recommended rates, paraquat (400-600 g a.i. ha^{-1}), glufosinate-ammonium (500 g a.i.ha^{-1}) and glyphosate (1000 g a.i. ha^{-1}) applied to field to control weeds in oil palm plantation, therefore leave no phytotoxic residue in the soil.

Key Words: Bioassay; Herbicide residues; Paraquat; Glyphosate; Glufosinate-ammonium; Field-treated plots

INTRODUCTION

Herbicides are used quite extensively in agricultural production system throughout the world, and Malaysian plantations are no exception to this. Paraquat, glufosinate-ammonium and glyphosate are most common herbicides used in these plantations. Herbicides, when applied to the field do not only control targeted weeds, but may also leave unwanted residues in the soil, which are ecologically harmful (Grossbard, 1972; Haney *et al*., 2000; Derksen *et al*., 2002; Riaz *et al*., 2007). Although the efficacy of herbicide in controlling weeds is important, its residual impact should also be considered for environmental safety. Preferred herbicides should not only have good efficacy, but also poses minimum adverse effects to crop, ecology and environment (Constenla *et al*., 1990; Hoerlein, 1994; Kamrin, 1997; Atkin & Leisinger, 2000; Anonymous, 2004; Faheed & Abd-Elfattah, 2007).

Bioassay and chromatography are among the several methods commonly used to determine pesticide residues in soil. Bioassay for residual phytotoxicity has been used to investigate many practical aspects of herbicide behavior in the environment for long time (Horowitz, 1976). It is a practical qualitative or semi-quantitative method of analysis, whereby the residues, which really affect plant development, are determined. Commonly, seed germination and seedling performance are used to investigate the residual phytotoxicity (Nyffeler *et al*., 1982; Sunderland *et al.*, 1991). The residual phytotoxicity effects can detect the herbicide or chemical residue present in the soil at concentrations high enough to adversely affect crop growth, yield and quality (Pestemer *et al*., 1980). Depending on the type and concentration of the residues, injury symptoms usually appear within 10 to 20 days after seedling emergence. Cucumber, sorghum, corn and oat are the common plant species used in bioassay studies (Horowitz, 1976), because they are easy to grow and have marked sensitivity to many herbicides.

Present study reports residual phytotoxicity effects of three commonly used broad spectrum herbicides, namely paraquat, glufosinate-ammonium and glyphosate.

MATERIALS AND METHODS

Experimental set-up and treatment. Field treatment was conducted in plots set-up in a young two-year old oil palm plantation of MAB Agriculture-Horticulture Sdn. Bhd., Sepang, Selangor, Malaysia. The experiment was laid out as randomized complete block design with four replications and each plot measured 5 x 20 m^2. The plots were sprayed at 200 L ha^{-1}, with four rates of each of paraquat (200, 400, 600 & 800 g a.i. ha^{-1}); glufosinate-ammonium (200, 400, 600 & 800 g a.i. ha^{-1}) and glyphosate (400, 800, 1200 & 1600 g a.i. ha^{-1}) and un-treated control plots. The herbicide formulations used were Gramoxone^R (200 g L^{-1} paraquat,

To cite this paper: Wibawa, W., R.B. Mohamad, A.B. Puteh, D. Omar, A.S. Juraimi and S.A. Abdullah, 2009. Residual phytotoxicity effects of paraquat, glyphosate and glufosinate-ammonium herbicides in soils from field-treated plots. *Int. J. Agri. Biol*., 11: 214–216

Syngenta Crop Protection), Basta 15^R (150 g L^{-1} glufosinate-ammonium, Bayer Cropscience) and Roundup^R (360 g L^{-1} glyphosate Monsanto). Knapsack sprayer fitted with AN 2.5 deflector nozzle was used for spraying.

Soil sampling and bioassay. Ten kg soil was taken randomly, at each sampling time of day 1 (DAT) and 1 and 2 weeks after treatment (WAT), from five spots in each plot to a depth of 10 cm and bulked together. A total of 400 g of the collected soil from each plot was placed in a plastic pot (9 cm diameter, 10 cm deep). Ten corn and cucumber seeds were planted at about 10 mm depth in each designated pot accordingly. The pots were then placed in the glasshouse for the bioassay study. Watering was done daily to saturation. Seed germination (seedling count) was recorded after five days and at which time the seedlings were thinned to three plants per pot for further studies.

The seedlings left for seedling development study were harvested at 2 weeks after sowing to determine residual effect in the soil. The growth components of the seedlings (plant height, leaf area, root length & total dry weight, as common assessment used in bioassay studies) were measured. Plant height was taken from soil surface to tip of the youngest leaf (for corn) and to point of growth (for cucumber). Leaf area was measured using LI-3100 Leaf Area Meter. Root length was taken after washing off the soil and measured from the point of growth to the tip of the longest roots. The seedlings were then placed in labeled envelopes accordingly, air-dried for 2 days before drying in the oven at 75^{o}C for 48 h to determine the dry weight. The effects of herbicide residues in soil were determined based on the plant responses through the growth components of the seedlings.

RESULTS AND DISCUSSION

Both the plant species showed similarity in their sensitivity to the residues in the soil. The effects on germination and seedling growth were comparable and consistent for both plants assayed on those soils sampled at 1 DAT and 1 and 2 WAT. Paraquat, glufosinate-amminium and glyphosate applied to the field at rates inclusive of their field recommended rates (400-600 g a.i. ha^{-1}, 500 g a.i.ha^{-1} & 1000 g a.i. ha^{-1}, respectively) did not affect the germination of seeds of these known sensitive plants used for the bioassay. The germination percentages of each crop from the soil of the treated plots at 1 DAT and 1 and 2 WAT were >90%, being insignificant to that percentage of the control plot (98%) (Table I). Results of the assessments of plant height, leaf area, root length and dry weight of corn and cucumber seedlings bioassayed for two weeks on the soil sampled at 1 DAT and 1 and 2 WAT also indicated that they were not affected by the residues of the herbicides treated at those rates. Therefore, using paraquat and glufosinate-ammonium (200-800 g a.i. ha^{-1}) and glyphosate (400-1600 g a.i. ha^{-1}), which include their field recommended rates, had no phytotoxic residues in the soil, thus causing no adverse effects to plant growth and development (Tables II & III).

Table I. Germination percentage of cucumber and corn seeds affected by herbicide residues in soil

[1]Herbicide (g a.i. ha^{-1})	Mean percent germination at days of soil sampling					
	Cucumber			Corn		
	1	7	14	1	7	14
Untreated	97.9	97.9	97.9	97.9	95.8	100
P 200	97.9	93.8	91.7	95.8	91.7	91.7
P 400	97.9	100	95.8	97.9	91.7	91.7
P 600	95.8	93.8	97.9	95.8	95.8	91.7
P 800	91.7	97.9	93.8	95.8	91.7	91.7
Ga 200	100	95.8	100	93.8	95.8	91.7
Ga 400	100	95.8	91	91	95.8	95.8
Ga 600	100	91.7	97.9	97.9	95.8	95.8
Ga 800	95.8	95.8	95.8	95.8	95.8	91.7
G 400	100	93.8	91.7	91.7	95.8	91.7
G 800	95.8	93.8	95.8	95.8	95.8	95.8
G 1200	97.9	95.8	97.9	93.8	95.8	91.7
G 1600	100	93.8	95.8	93.8	95.8	100
[2]Mean comparison	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1]P=paraquat; Ga=glufosinate-ammonium; G=glyphosate; [2]Mean comparisons are non-significant (n.s.) at $P<0.05$ among herbicides, rates within herbicide and with untreated control

The fact that paraquat, glufosinate-ammonium and glyphosate are post-emergent herbicides, it is understandable that their effect on seed germination should be minimal or zero. However, if the residues were to be present at phytotoxic level, the effect on seedling development should be expressed by those seedlings. Adverse effect of herbicide residues in soil could be determined with plant response (Pestemer *et al*., 1980). In present study, paraquat, glufosinate-ammonium and glyphosate did not affect seedling growth of corn and cucumber in relation to their plant height, leaf area, root length and accumulation of dry weight, thus indicating absence of residue or the residues were un-available to the plants. Streibig (1984 & 1988) indicated that when indicators of plant growth were plotted against logarithm of herbicide dose, they were common to find a symmetrical sigmoid relationship. Since the plant growth parameters among different treatment doses did not show significant different, thus a symmetrical sigmoid curve of the dose-response curve was not obtained. Pestemer *et al*. (1980) classified indicator of plant responses to residual herbicides into the activity categories of stimulation (100% safe), no response (90-100% safe), slight damage (70-90%, slight risk), moderate damage (50-70%, significant damage) and severe damage (0-50%, crop failure). Thus, treatment with paraquat, glufosinate-ammonium and glyphosate at rates even higher than their field recommended rates gave an indicator response showing the treatments were safe (>90% response) and did not cause adverse effect to the growth components of the plants.

In conclusion, paraquat, glufosinate-ammonium and glyphosate, when applied to the field at rates with ranges inclusive of their field recommended rates did not leave residues in the soil, which may cause phytotoxic effect to the indicator plants, corn and cucumber. Germination rates

Table II. Plant height and root length of cucumber and corn seedlings affected by herbicide residues in soil

[1]Herbicide (g a.i. ha^{-1})	Mean plant height (cm) at days of soil sampling						Mean root length (cm) at days of soil sampling					
	Cucumber			Corn			Cucumber			Corn		
	1	7	14	1	7	14	1	7	14	1	7	14
Untreated	9.5	9.7	10.1	35.2	35.8	37.4	7.7	7.9	8.2	20.1	20	20.3
P 200	9.4	8.9	9.6	33.1	37	36.6	7.5	7.1	7.4	18.3	20.8	19.3
P 400	8.8	9.5	9.8	35	36.3	37	7.1	7.5	8	18.9	19.8	20.5
P 600	9.1	10.1	9.7	34.4	34.8	34.6	7.3	7.9	7.6	18	18.8	20
P 800	10.1	9.5	10.9	34.1	33.8	34.1	7.9	7.6	8.7	21.6	20.3	19.5
Ga 200	9.6	9.9	10.3	33.4	35.8	36.1	7.6	7.7	8.2	18.3	19.8	19.3
Ga 400	10.1	10.2	11	36.9	37.1	37.9	7.7	7.9	8.3	17.6	19.3	19.5
Ga 600	9.5	10	10.9	36	36.4	35.3	7.3	7.7	8.4	19.6	20.5	19.5
Ga 800	9.5	10.1	10.5	33.8	35.5	35.9	7.5	7.7	8	20.4	20.8	19.9
G 400	9.9	9.6	10.6	34.9	36.4	36.6	7.5	7.4	8.2	18.5	19.8	20.5
G 800	9.6	10.1	11.1	34.4	36.3	33.4	7.5	7.9	8.2	20.8	20.3	20.3
G 1200	10.1	10.1	10.9	35	34	38.8	8	8.1	8.4	18.6	20.3	20.3
G 1600	9.6	10.2	10.6	36.5	36.3	38.1	7.4	8	8.1	20.1	19.8	20.5
[2]Mean comparison	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1]P=paraquat; Ga=glufosinate-ammonium; G=glyphosate; [2]Mean comparisons are non-significant (n.s.) at P<0.05 among herbicides, rates within herbicide, and with untreated control.

Table III. Total leaf area and dry weight of cucumber and corn seedlings affected by herbicide residues in soil

[1]Herbicide (g a.i. ha^{-1})	Mean total leaf area (cm^2) at days of soil sampling						Mean total dry weight (mg plant^{-1}) at days of soil sampling					
	Cucumber			Corn			Cucumber			Corn		
	1	7	14	1	7	14	1	7	14	1	7	14
Untreated	12.2	11.6	12.3	27.6	27.3	32.2	44	53	51	121	148	172
P 200	11.5	10.6	11.2	27.5	24.1	30.9	41	49	46	127	156	155
P 400	11.9	10.2	11	25.4	26.6	30.6	42	48	46	132	151	155
P 600	12.2	12.6	11.1	26.5	24.5	28.3	44	48	47	130	134	156
P 800	11.1	10.5	11.9	25.1	24.9	27.3	40	51	47	130	156	156
Ga 200	11.3	12.5	11.6	27.6	24.9	28.1	47	51	48	127	149	160
Ga 400	10.9	11.4	11.1	27.3	26.3	31.7	45	56	52	130	138	154
Ga 600	12.1	11.3	11.3	27.1	27.1	28.5	45	52	50	128	155	158
Ga 800	11.6	10.7	11.5	25.1	27.1	28.1	45	50	53	122	159	153
G 400	12	12.5	12.4	28.9	26.1	30.5	45	48	46	130	131	183
G 800	11.1	11.6	12.3	26.9	28.9	28.5	44	48	47	130	155	155
G 1200	11	11.6	11.2	24.7	26.7	32.7	47	49	48	126	155	156
G 1600	11.9	12.4	11.5	25.6	27	29.2	43	49	46	129	150	155
[2]Mean comparison	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

and seedling development of the indicator plants were more than 90 percent normal (when compared with those from un-treated plots).

Acknowledgements. The authors thank Faculty of Agriculture, University Putra Malaysia and MAB Agriculture-Horticulture Sdn. Bhd. for assistance in the conduct of this work.

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(Received 03 November 2008; Accepted 04 December 2008)