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Determination of the Leaching Potential and Residues Activity of Imidazolinone Herbicide in Clearfield Rice Soil Using High-Performance Liquid Chromatography

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Abstract

The residual activity of herbicides may be detrimental to the environment, requiring analysis of the persistent residues in the soil and water. A field study was conducted to measure the residues of Imidazolinone (IMI) in three Clearfield® rice field soils at three different locations in Malaysia. The analyses of IMI in the soil samples were carried out using a high-performance liquid chromatography (HPLC). These herbicides are widely used; however, few studies have been conducted on both residues, especially in the context of Malaysian soil. Residues of imazapic and imazapyr were found to fall within 0.03–0.58 µg/mL and 0.03–1.96 µg/mL, respectively, in three locations. IMI herbicides are persistent in the soil, and their residues remain for up to 85 days after application. A pre-harvest study was suggested for these herbicides on water, which will provide a clearer indicator on the use of IMI in Clearfield® rice fields.

Keywords Chromatography · Herbicides · HPLC · Imazapyr · Imazapic

IMI herbicide usage is regarded as one of the main strategies of controlling weedy rice population. Imazapyr and imazapic 2-(4-isopropyl-4-methyl-5-oxo-2-imidazoline-2-y1) nicotinic acid] and [2-(4-isopropyl-4-methyl-5-oxo-2-imidazoline-2-yl)-5-methylnicotinic acid]) interrupts the production of branched amino acids by inhibiting acetohydroxyacid synthase (Ureta et al. 2017). Both are broad-spectrum IMI herbicides that control many kinds of grass and broadleaf weeds (Ottis et al. 2004). Weedy rice is considered as a major agricultural threat and one of the most damaging global weeds (Bzour et al. 2018). Due to the diversity of weedy rice in Malaysia (Song et al. 2014), the decrease in rice yields caused by weedy rice in Malaysia was reported to be 30–50% from the total rice yield (Watanabe et al. 2000). Recently, the Ministry of Agriculture introduced a new rice cultivation technology known as the Clearfield® Production System (CPS), which uses the Clearfield® rice (MR 220CL1 and MR 220CL2) and a herbicide called OnDuty®, which contain both imazapic and imazapyr herbicides. Clearfield® rice is considered as an effective tool for controlling and mitigating the propagation of weedy rice in a cultivated rice field. CPS technology helps rice croppers mitigate the aggressiveness of weedy rice and improve the growth of rice agriculture in Malaysia.

However, farmers and researchers reported that IMI was unable to fully eradicate the weedy rice. There are also many reports stating that the IMI herbicide carryover influences many non-rice crops in rotational systems (Alister and Kogan 2005). Currently, the massive use of IMI herbicides resulted in increased resistance to weedy rice (Burgos et al. 2014). Additionally, IMI residues may be adsorbed or leached at different levels, and controlled by the physicochemical properties of the soil, encompassing solubility, organic matter, and the pH of the soil. The rainfall index is an important factor for determining herbicide leaching. These herbicides were studied in the previous decade to determine the risk it poses to the environment, water, and

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soil. (Bajrai et al. 2017; Marcia 2014). High-performance liquid chromatography (HPLC) is a powerful technique that can be used to separate and analyze analytes in a solution. There is a need here to devise methods capable of providing reliable analytical data onto residues and to monitor these compounds in soil (Martins et al. 2014).

Currently, studies on IMI residues in soil (in the sub-tropical region, especially in Malaysia), remain scarce. The use of CPS technology in Malaysia goes back six years, and a wide range of crops, especially rice, are repeatedly treated with IMI. The present study was conducted to develop a reliable analytical method to measure the contamination levels of imazapic and imazapyr in the soils of three paddy fields.

Materials and Methods

A study was meticulously planned to determine terminal residues of IMI in the soil. The soil samples were taken in November 2016 from three Clearfield rice fields in Sawah Sempadan-Tanjung Karang district. The farmers' field was located at (3°25′35.0724″, E101°10′36.1704″) in Kuala Selangor, Malaysia. The physicochemical characteristics of the soil were determined for the three fields, as tabulated in Table 1. The region experiences a sub-tropical climate, with almost high daily rainfall and temperatures. IMI herbicides have been used in this area for the past six years. Twenty samples were collected randomly prior to harvesting the crops. The samples were directly stored in a sterile zip-lock polyethylene bag and coded using a special waterproof sticker. On the same day, the samples were placed in a special room at 35°C under the shade for up to 5 days, then, the dried samples were ground and sieved via stainless steel sieve (2 mm) and stored in a refrigerator (at 4°C).

Herbicide standards were purchased from Sigma-Aldrich (USA), with purities of 95.5% and 99.9% for imazapic and imazapyr, respectively. Methanol, dichloromethane (DCM), and acetonitrile 99.9% (HPLC gradient) (Fisher), acetic acid, ACS reagent (Fisher), formic acid, 98% (EM Science), and all materials for the HPLC experiments were purchased from Sigma Aldrich (Germany). Ultrapure water obtained from a Milli-Q Direct UV3® system (Millipore, USA), was also filtered through a 0.2 µm Whatman filter paper. Other equipment includes a DuPont Sorvall Centrifuge (Model RC-5C), centrifuge bottles with cap 45 mL polypropylene (kontes Scientific), vortex mixer (Lambert 3000), and Supelco SPE cartridges.

The soil samples were analyzed using a simple modified extraction method proposed by (Krynitsky et al. 1999; Ramezani et al. 2009). In this procedure (5 ± 0.001 g) of randomly homogenized soil sample was weighed and placed in a 250-mL centrifuge tube, followed by the addition of ~ 150 mL of 0.5 N NaOH. The samples were stored, for 45 min in an end-over-end shaker at 30°C to allow for equilibration. Ten ml of methanol was added to the precipitate of humic acid, followed by sonicating the samples for 10 min and were centrifuged for 10 min (at 7000 rpm) to remove particulates. The solution was filtered and adjusted to a (pH 2.0) using 6 N HCl. The suspension was left at room temperature prior to analysis, where the sample solution was transferred to a 500-mL separatory funnel and extracted using 50 ml dichloromethane twice, then mixed and transferred to the flask. Dichloromethane was dried using anhydrous Na_2SO_4, and the solution was passed through a smooth activated charcoal column. The resulting solution was evaporated at 65°C to near dryness. The residue was diluted using ~ 2 mL of methanol: 0.1% formic acid (1:1), then DSC-18-6 mL cartridge 500 mg conditioned with 3 mL of each of the methanol, acetonitrile, and H_2O. The sample was then loaded through the cartridge under vacuum. The analytes were washed using 9 mL H_2O and 6 mL (60/40) (H_2O: acetonitrile). Finally, the vials were placed in a vacuum and the cartridge eluted with 3 Ml methanol: 0.1% formic acid solution. The sample extracts were then filtered through a 0.2 µm polytetrafluoroethylene membrane, transferred to a 1.5 mL HPLC autosampler vial, and stored at 4°C until the HPLC analysis. IMI standard solutions were individually prepared in acetonitrile at concentrations of (100 µg/mL), respectively, by dilution from a 1000 µg/mL stock solution. Afterward, other freshly diluted standard solutions were prepared in acetonitrile. All stocks and working solutions were stored at − 18°C in dark conditions (Marcia 2014).

IMI residues were analyzed using an HPLC–UV system consisting of Shimadzu high-performance liquid

Table 1 The physicochemical characteristics of the soil at three locations of the study area

Location	Depth (cm)	PH	Moisture%	Sand%	Silt%	Clay%	OM%	Soil type[a]
Field A	0–20 20–40	6.21 6.70	38.0 30.3	39.0	29.0	30.0	2.0	Clay loam
Field B	0–20 20–40	6.81 6.61	44.0 57.0	24.6	35.7	39.2	1.3	Clay loam
Field C	0–20 20–40	7.10 6.94	38.0 59.0	25.0	35.0	38.0	1.9	Clay loam

OM organic matter

[a]Soil type according to soil texture triangle

chromatography with LC-10AT pump and SPD- 20A interfaced with LC software. The HPLC column used was Thermo $-C_{18}$ (4.6 × 250 mm^2, µm) (USA). The gradient solvent program used mobile phase A (acetonitrile 100%) and mobile B (water, including 0.1% of acetic acid) (pH 2.8). The initial gradient program was: 30% A (0–1 min), 30%–45% (1–5 min), and 45%–35% (5–13 min). A "17 µg" aliquot of the samples were injected into the column. Linearity calibration curves were constructed using different standard concentrations (0.1, 0.5, 1, 5, 10, and 20 µg/mL). The concentrations of both IMI herbicides were determined by comparing the peak area of the samples that deduced from the calibration curve. The spiked soil samples were fortified with standard solutions (0.1, 0.5, 5, 10 µg/mL).

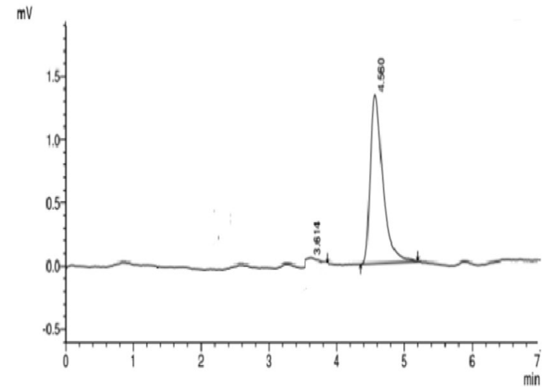

Fig. 2 Imazapyr standard 0.5 µg/mL

Results and Discussion

Calibration curves from different known concentrations of imazapic and imazapyr herbicides (0.1, 0.5, 1.5, 10, 20 µg/mL) were constructed, as shown in Table 2. The equations of analytical calibration graphs, obtained by plotting peak areas against concentrations of the imazapic and imazapyr herbicides. The linear regression equations were y = 64,086x + 6626.7, with R = 0.9978 for imazapic, and y = 35078X + 3189.9, with R of 0.9998 for imazapyr respectively, good linearity is showed in Figs. 1 and 2.

Twenty samples were collected from three field sites in Sawah Sempadan-Tanjung Karang district and analyzed using the aforementioned procedure. The results were included in Table 3.

To study the recovery of both herbicides, samples were fortified with different concentrations of standard solutions of each herbicide. The results were presented in Table 4 below.

Previous researches reported that these herbicides are slow to degrade in soil under normal environmental conditions (Bajrai et al. 2017). Imazapyr has a half-life of 90–120 days, while imazapic has a half-life of 3 months. The K_{oc} for both herbicides were 137 and 100 mL g^{-1}, respectively, which means low adsorption and high mobility, and eventually the high level of leaching. Nevertheless, these herbicidal residues persist for extended periods of the times, thus representing a high risk of environmental contamination of soil, surface, and groundwater, especially imazapic (Souza et al. 2016). The LOD and LOQ were found to be 1.04 and 4.09 µg/mL for imazapic, and 0.17 and 0.51 µg/mL for imazapyr, respectively as found from our previous study (Bzour et al. 2017). From Table 3, imazapic and imazapyr were found at depths more 20 cm. The residues were at 0.58, 0.03 on the first 20 cm and 1.96, 0.58 at the 20–40 cm depth, which agrees with (Neto et al. 2017; Refatti et al. 2017), who reported that imazapyr and imazapic can leach up to more than 25 cm. The presence of both IMI residues at 20–40 cm depth in field 1 (plot-1) may be due to the soil sample location at the edge of the field (on the corner of the field), and it is the first sample collected. The activities, practices and procedures of the farmers are instruments towards the presence of these herbicides. Some plots were not cultivated, and seldom plowed, which may result in reduced uptake of sunlight, and accumulation of IMI residues throughout the seasons. Table 3 shows that the imazapic residues were present in most samples, especially at depths of more than 20 cm, in contrast to imazapyr residues, which were only found in the field 1–plot 1 and field 3–plot 2. This could be attributed to the concentration of imazapyr and imazapic in the whole compound (Onduty® compound were 0.58 and 0.19 g/L, respectively). Therefore, the concentration of imazapic is tripled, which could explain the accumulation and translocation of imazapic more than imazapyr. (Vizantinopoulos and Lolos 1994) pointed out that imazapyr has low persistence, and can move and leach into deep layers, reaching more than 45 cm. The residues of imazapic in the plots decreased from soil depths of 2040 cm. The residues

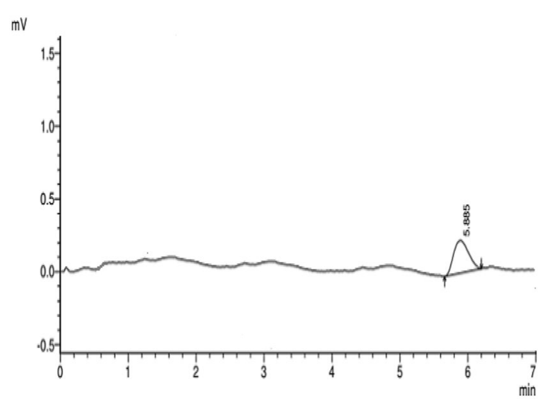

Fig. 1 Imazapic standard 0.1 µg/mL

Table 2 Peak area versus concentration (0.1 to 20 μg/mL) for imazapic and imazapyr

Concentration (μg/mL)	Area for imazapic[a]	Std. deviation	Area for imazapyr	Std. deviation
0.1	3477	±124.02	3680	±140.00
0.5	54,956	±5512.72	21,381	±949.65
1	59,366	±8092.74	39,914	±4501.50
5	305,898	±65,193.93	179,150	±8560.39
10	688,046	±96,563.22	354,730	±36,051.21
20	1,273,564	±178,045.52	704,156	±2816.62

Std deviation standard deviation

[a]Average of three replications

in plot 1 were 0.58, 0.03, plot. 2: 0.03 (–), plot 3: (–), plot 4: 0.10, plot 5: 0.04 (–) and Plot 6 were 0.09, 0.05 μg/mL, respectively. The reason for the shift down of the peak areas could be due to translocation involving the movement of soil–forming materials throughout the soil's profile and the leaching of herbicides into deeper layers.

The adsorption of herbicides decreased due to increasing heavy rain and temperatures. The higher solubility in water, pH, high temperatures, and high rainfall in Malaysia are some of the main factors that play an important role in the transition of residual particles of herbicides through the pores or movement to deeper layers, as per the studies reported by (Castillo et al. 1997; Fish et al. 2015; Grey et al. 2012). Malaysia has almost daily high-intensity rainfall and medium daily temperatures. Studies reported that temperatures between 35 and 45°C and increased soil moisture enhances both chemical and microbial degradation of herbicides, as well as their respective mobilities (Jourdan et al. 1998; Laabs et al. 2000; Neto et al. 2017). Therefore, different factors can affect the leaching of these types of herbicides into the depth of the soils, including the pH, concentration of herbicides, and type of the soil. At pH values greater than 6, the IMI herbicides are weakly adsorbed into the soil (Ozcan et al. 2017). Another important factor that affects the residual concentration is the type of the soil. IMI sorption increases alongside soil clay content, due to increased bindings of the herbicide to soil particles (Gianelli et al. 2014). The type of the soil is clay loamy, which means that the percentage of adsorption increase and IMI herbicides dissipation decrease. Sondhia (2013) reported that IMI-herbicides could leach into clay loam soil up to a depth of 70 cm.

Conclusion

A reliable method for the identification of IMI herbicides in Clearfield rice soils, has been developed. HPLC–UV was used for this purpose, and the linearity, accuracy, and recovery data were determined. The LOD and LOQ were found to be 1.04 and 4.09 for imazapic and 0.17 and 0.51 μg/mL for imazapyr, respectively. The results showed that residual herbicides were present in the soil in certain plots, reaching 20–40 cm. It was observed that high mobility herbicides can leach into deeper layers of the soil, which could threaten deep aquifers. This study elucidated the environmental properties of IMI herbicides that are commonly used in major crops, such as rice. The results also confirmed the need for more in-depth studies at different times of application, to precisely evaluate the actual leaching depth of these herbicides and its mechanisms.

Table 3 Terminal residues of the imazapic and imazapyr (μg/mL) at various locations for the different samples

Fields	Plot	Sample	F. C	Depth (cm)	R.T for imazapic	Area for imazapic[a]	Determined Concentration for imazapic	R.T for imazapyr	Area for imazapyr[a]	Determined Concentration for imazapyr
Field 1	1	A	F1P1D1	0–20	5.8	64,276	<LOQ	4.6	84,945	1.96
		B	F1P1D2	20–40	5.9	1091	<LOQ	4.6	21,381	0.58
	2	A	F1P2D1	0–20	5.6	16,879	<LOQ	4.6	1079	<LOQ
	4	A	F1P4D1	0–20	5.7	5026	<LOQ	–	–	–
	5	A	F1P5D1	0–20	5.8	1608	<LOQ	–	–	–
	6	A	F1P6D1	0–20	5.7	4871	<LOQ	–	–	–
		B	F1P6D2	20–40	5.9	2318	<LOQ	–	–	–
Field 2	1	A	F2P1D1	0–20	5.7	3353	<LOQ	–	–	–
		B	F2P1D2	20–40	5.6	2214	<LOQ	–	–	–
	2	A	F2P2D1	0–20	5.8	7541	<LOQ	4.6	5421	<LOQ
Field 3	2									
		A	F3P2D1	0–20	6.0	1562	<LOQ	4.5	56,983	<LOQ
		B	F3P2D2	20–40	–	–	–	4.8	1326	<LOQ

F.C field code, *R. T* retention time

[a]Average peak area represent the average peak areas value of same plot soil samples. Limit of quantitation (LOQ) for imazapic and imazapyr (4.09 μg/mL) and (0.51 μg/mL) respectively The fields which did not contain IMI residues were removed

Table 4 Recovery of imazapic and imazapyr from the soil

Fortified concentration (µg mL)	Recovery (%) for imazapyr[a]	Av. recovery for imazapyr ± SD	Recovery (%) for imazapic	Av. recovery for imazapic ± SD
0.1	(96.4; 110.9; 114.0)	107.0 ± 9.4	(71.9; 70.9; 70.6)	71 ± 0.6
0.5	(72.6; 80.1; 89.5)	80.7 ± 8.4	(52.5; 82.8; 102.0)	79 ± 20.4
5	(79.2; 77.9; 73.1)	76.7 ± 3.1	(97.4; 97.4; 77.6)	90 ± 11.2

(n = 3)

SD standard deviation

[a]The averages of three samples processed through the procedure

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Alister C, Kogan M (2005) Efficacy of imidazolinone herbicides applied to imidazolinone-resistant maize and their carryover effect on rotational. Crop Prot 24:375–379. https://doi.org/10.1016/j.cropro.2004.09.011

Bajrai F, Ismail B, Mardiana-Jansar K, Omar R (2017) Persistence of imazapic and imazapyr in paddy soil and water. Int J Adv Environ Eng 4:12–15

Burgos NR, Singh V, Tseng TM, Black H, Young ND, Huang Z, Caicedo AL (2014) The impact of herbicide-resistant rice technology on phenotypic diversity and population structure of United States weedy rice. Plant Physiol 166:1208–1220. https://doi.org/10.1104/pp.114.242719

Bzour MI, Zuki FM, Mispan MS, Mohamed KA, Jodeh SW, Monzir SA (2017) A simple method for determination and characterization of imidazolinone herbicide (imazapyr/imazapic) residues in clearfield® rice soil. Appl Ecol Env Res 4:891–902

Bzour MI, Zuki FM, Mispan MS (2018) Introduction of Imidazolinone Herbicide and Clearfield® Rice Between Weedy Rice Control Efficiency and Environmental Concerns (Residues/Resistance): A Review. Environ Rev. 26(2):181–198

Castillo LE, de la Cruz E, Ruepert C (1997) Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. Environ Toxicol Chem 16:41–51. https://doi.org/10.1002/etc.5620160104

Fish JC, Webster EP, Blouin DC, Bond JA (2015) Imazethapyr co-application interactions in imidazolinone-resistant rice. Weed Technol 29:689–696

Gianelli VR, Bedmar F, Costa JL (2014) Persistence and sorption of imazapyr in three Argentinean soils. Environ Toxicol Chem 33:29–34. https://doi.org/10.1002/etc.2400

Grey TL, Cutts GS III, Johnson J (2012) Imidazolinone-resistant soft red winter wheat weed control and crop response to ALS-inhibiting herbicides. Weed Technol 26:405–409

Jourdan SW, Majek BA, Ayeni AO (1998) Imazethapyr bioactivity and movement in soil. Weed Sci 46:608–613

Krynitsky AJ, Stout SJ, Nejad H, Cavalier TC (1999) Multiresidue determination and confirmation of imidazolinone herbicides in soil by high-performance liquid chromatography/electrospray ionization mass spectrometry. J AOAC Int 82(4):956–962

Laabs V, Amelung W, Pinto A, Altstaedt A, Zech W (2000) Leaching and degradation of corn and soybean pesticides in an Oxisol of the Brazilian Cerrados. Chemosphere 41(9):1441–1449

Marcia EA (2014) Amethod for determination of imazapic and imazethapyr residues in soil using an ultrasonic assisted extraction. Bull Environ Contam Toxical 93:360–364

Martins GL, Friggi CA, Prestes OD, Vicari MC, Friggi DA, Adaime MB, Zanella R (2014) Simultaneous LC–MS/MS determination of imidazolinone herbicides together with other multiclass pesticide residues in soil. Clean-Soil Air Water 42:1441–1449. https://doi.org/10.1002/clen.201300140

Neto MDdC, Souza MdF, Silva DV, Faria AT, da Silva AA, Pereira GAM, de Freitas MAM (2017) Leaching of imidazolinones in soils under a clearfield system. Agron Soil Sci 63:897–906

Ottis BV, O'barr JH, Mccauley GN, Chandler JM (2004) Imazethapyr is safe and effective for imidazolinone-tolerant rice grown on coarse-textured soils. Weed Technol 18:1096–1100

Ozcan C, Cebi UK, Gurbuz MA, Ozer S (2017) Residue analysis and determination of imi herbicides in sunflower and soil by GC–MS. Chromatographia 80:941–950

Ramezani M, Simpson N, Oliver D, Kookana R, Gill G, Preston C (2009) Improved extraction and clean-up of imidazolinone herbicides from soil solutions using different solid-phase sorbents. Chromatography A 1216:5092–5100

Refatti JP, Avila LAd, Noldin JA, Pacheco I, Pestana RR (2017) Leaching and residual activity of imidazolinone herbicides in lowland soils. Ciência Rural 47:5

Sondhia S (2013) Evaluation of imazethapyr leaching in soil under natural rainfall conditions. IJWS 45:58–61

Song BK, Chuah TS, Tam SM, Olsen KM (2014) Malaysian weedy rice shows its true stripes: wild Oryza and elite rice cultivars shape agricultural weed evolution in Southeast Asia. Mol Ecol 23:5003–5017. https://doi.org/10.1111/mec.12922

Souza M, Neto M, Marinho M, Saraiva D, Faria A, Silva A, Silva D (2016) Persistence of imidazolinones in soils under a clearfield system of rice cultivation. Planta Daninha 34:589–596

Ureta M, Carbonell FT, Pandolfo C, Presotto A, Cantamutto M, Poverene M (2017) IMI resistance associated to crop-weed hybridization in a natural Brassica rapa population: characterization and fate. Environ Monit Assess 189:101

Vizantinopoulos S, Lolos P (1994) Persistence and leaching of the herbicide imazapyr in soil. Bull Environ Contam Toxical 52:404–410

Watanabe H, Vaughan D, Tomooka N (2000) Weedy rice complexes: case studies from Malaysia, Vietnam, and Surinam Wild and Weedy Rice in Rice Ecosystems in Asia: a review. IRRI 10:25–34