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Imazaquin degradation and metabolism in a sandy loam soil amended with farm litters

WANG Hui-li, LI Yan-yan, WEI Guo-hui, WANG Xue-dong*

Key Laboratory of Pesticide and Chemical Biology of Ministry of Education, Central China Normal University, Wuhan 430079, China. E-mail: zjuwxd@yahoo.com.cn

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Abstract

Imazaquin applied in legume crops has a long residual time in soil, which often impacts safety of the susceptible crops. To increase safety of imazaquin application, two composted litters, bovine manure (BM) and chicken manure (CM), were used to determine their effects on imazaquin environmental behavior by incorporating each kind of manure into the tested sandy loam soil at 10% (w/w). The degradation of imazaquin in BM- and CM-amended soil was about 2.4 and 1.5 times, respectively, faster than that in unamended soil. The half-lives of imazaquin in BM-amended soil varied between 6.7 and 15.4 d over the temperature range of 20 to 40°C, and the degradation rate constant (k) increased by a factor of about 1.5 for every 10°C change. Higher mix ratio did not significantly increase the degradation, and the optimal active degradation of imazaquin was observed approximately at the mix ratio of 10:1 of soil to BM. The different moisture levels had negligible effect on imazaquin degradation. In both unamended and BM-amended treatments, two metabolites were observed at 5, 10 and 30 d after treatment. One metabolite at retention time (RT) of 8.4 min was identified as 2-(4-hydroxyl-5-oxo-2-imidazolin-2-yl) quinoline acid, originating from the loss of isopropyl group and hydroxylation at the 4-position of imidazolinone ring. The other at RT of 12.9 min was identified as quinoline-2,3-dicarboxylic anhydride, resulting from detachment of imidazolinone ring and the forming of dicarboxylic anhydride. This finding suggested that the addition of farm litters into soil might be a good management option since it can not only increase soil fertility but also contribute to increase safety of imazaquin application to the following susceptible crops.

Key words: imazaquin; farm litter; enhanced degradation; metabolite

Introduction

Soil contamination caused by the use of pesticides and its adverse effects are major problems that we are facing today. Among several types of treatment methods for contaminated soils, the use of organic residues has advantages, such as relatively low cost, simplicity of operation and design, and relatively high treatment efficiency (Freeman and Harris, 1995). Readily degradable organic matters such as manures, yard wastes, and food-processing wastes are often added to supplement the nutrients to soil. Farm litter is commonly applied to the soil before application of pesticides. It contains a large number of microorganisms as well as various nutrients (nitrates and phosphates), and thus can accelerate the bioremediation of contaminated soil. The addition of farm litter frequently affects the rate and pathway of pesticide degradation in soils (Houot *et al*., 1998; Namkoong *et al*., 2002). Decreased bioavailability due to increased sorption to the additional organic matter of farm litter will retard degradation. On the other hand, co-metabolic biotransformation can be enhanced by the general increase in microbial activity stimulated by the organic matter of farm litter (Barriuso *et al*., 1997). Therefore, the mechanism for the effect of farm litter on pesticide degradation is complicated.

Imazaquin is a selective imidazolinone herbicide used for broad-spectrum weed control in soybean and other legume crops. It is an amphoteric molecule that has both an acidic carboxyl and a basic quinoline functional group with pK_a values of 3.8 and 2.0, respectively. Imazaquin is moderately water-soluble with solubility of 98 and 149 mg/L at pH 4 and 8, respectively. It breaks down very slowly by hydrolysis (Barkani *et al*., 2005) and is mobile (Milanova and Grigorov, 1996), which result in the potential to leach to groundwater and raise concern about its safety to human health. Additionally, the long residual time of imazaquin in legume crops would impact the following susceptible crops such as barley, cotton, corn or oats. Therefore, rapid elimination of imazaquin from the site of application or contamination assumes a vital importance for a safe environment. This herbicide was registered in mainland China in 1997, and has been extensively applied in many districts of China, especially in the northeast. The

Project supported by the Natural Science Foundation of Hubei Province (No. 2006ABA182). *Corresponding author.
E-mail: zjuwxd@yahoo.com.cn.

environmental behavior of imazaquin is known in soils (Gennari *et al*., 1998) and waters (Troiano *et al*., 2001). For example, imazaquin phototransformation studies in aqueous solution suggested that it degraded slowly in pure water and photolysis led to the formation of various photoproducts (Barkani *et al*., 2005). Another study of photosensitized degradation indicated that the photocatalytic effect was more efficient in an aqueous suspension of titanium dioxide containing 200 mg/L imazaquin. (Garcia and Takashima, 2003). So far, few study was concerned in the effect of organic manure on imazaquin environmental behavior, which reported that two amendments affected sorption and leaching of imazaquin in soils (Undabeytia *et al*., 2004). However, no data are available on the effect of farm litter on imazaquin degradation and metabolism in soils. The objective of this study was to determine if two litters, composted bovine and chicken manure, could be used to promote the degradation of imazaquin residue that might leach to groundwater or be inhibitory to a following crop.

1 Materials and methods

1.1 Materials

Technical grade imazaquin (97.5% purity) was procured from Shenyang Chemical Engineering Institute, Shenyang, China. The stock standard solution (200 μg/ml in methanol) was freshly prepared every month and stored in dark bottle at –20°C until use. Dichloromethane and methanol used in this study were of HPLC-grade (Tianjin Chemical Reagent Factory, Tianjin, China). The other analytical grade reagents and solvents locally obtained were purified and distilled before use. The water used was purified with a Mill-Q-Plus system.

1.2 Soil and farm litters

The soil was sampled from an experimental plot located at Pinghu, Zhejiang Province, Southeast China. The plot had been cultivated with maize continuous from 2000 to 2004, and maintained without vegetation for more than two years. The plot has never been applied with imazaquin. Physicochemical characteristics of the investigated soil were determined by standard methods. The soil pH was determined in a 1:1.25 of soil:water suspension using a glass electrode, and the mechanical fractions by the hydrometer method. Organic carbon (OC) content was determined according to the previous report (Walkley and Black, 1984), and organic matter (OM) content was calculated as follows: OM (%) = 1.724 × OC (%). The content of sand, silt and clay of soil were 56.7%, 24.4% and 18.9%, respectively, and the texture of the soil was classified as a sandy loam soil (Table 1). The collected soil was air-dried, ground and passed through a 2-mm sieve before use.

Two farm litters, composted bovine manure (BM) and chicken manure (CM), were collected from a farmhouse, located at the suburb of Zhengzhou City, China. The collected farm litters were air-dried at room temperature (25°C), homogenized, and crushed to pass a 4-mm sieve. The characteristics of the amendments are listed in Table 1. Microorganisms were numbered by plate counting on nutrient Agar for bacteria and Rose Bengal Agar for fungi (Houot *et al*., 1998), and the results are shown in Table 2.

Table 2 Plate counts of bacteria and fungi in experimental material

Type of material	Bacteria ($\times10^6$ CFU/g)	Fungi ($\times10^4$ CFU/g)
Sandy loam soil	5.6±0.7	3.1±1.2
Bovine manure (BM)	75.3±9.4	21.2±1.8
Chicken manure (CM)	17.4±2.1	13.5±1.5

*CFU = colony forming unit; ±SD (standard deviation, n=3).

1.3 Effect of farm litter on degradation

Each kind of farm litter was incorporated into the soil at 10% (w/w) for comparative purpose. The amended soil was mixed thoroughly, and spiked with imazaquin stock solution to obtain a final concentration of 10 μg/g on a dry weight basis. The treated soil was adjusted to 60% of maximum water-holding capacity (WHC_{max}). Aliquots (30 g) of the soil were introduced into the flasks, which were then weighed and incubated at 30±1°C in the dark. The weight loss due to evaporation of soil moisture was maintained by periodical addition of sterilized deionized water at intervals of 5 d over the whole incubation period. At specific time intervals of 0 (2 h after spiking), 5, 10, 20, 30, and 50 d after treatment (DAT), the samples were removed and processed for analyses of imazaquin residues. Soil without addition of either farm litters served as control, and each treatment including control was set up in triplicate.

1.4 Effects of temperature, moisture and amendment rate on imazaquin degradation

For temperature effect, the soil, amended with 10% (w/w) of BM and maintained at 60% of WHC_{max}, was incubated at 20, 30 or 40°C. With respect to moisture effect, the soil, amended with 10% (w/w) of BM and incubated at 30°C, was maintained at 30%, 60% and 80% of WHC_{max}. As for the effect of amendment rate, the soil,

Table 1 Basic physicochemical properties of the tested soil and farm litters

Type of material	pH	Organic matter (g/kg)	CEC (cmol(+) /kg)	TN (g/kg)	WHC_{max} (%)
Sandy loam soil	7.3	18.5	11.6	3.6	58.7
Bovine manure (BM)	7.8	478.3	48.9	16.4	49.8
Chicken manure (CM)	8.3	368.9	38.7	12.9	43.6

CEC: cation exchange capacity; TN: total nitrogen; WHC_{max}: maximum water-holding capacity.

adjusted to 60% of WHC_{max} and incubated at 30°C, was fortified at 5%, 10% or 15% (w/w) of BM. Each treatment in triplicate was then treated as described below in Section 1.5. The effect of each factor on imazaquin degradation was analyzed by comparing their respective degradation rate under different conditions.

1.5 Extraction and clean-up of soil sample

An aliquot (10 g) of each treatment sample (30 g) was spiked with 30 ml extraction solution (methanol:water, 70:30 by volume), which was acidified to pH 3–5. The mixture was shaken vigorously for 1 h on a mechanical shaker, and filtered under vacuum with repeated washing using methanol. The methanol was evaporated from the filtrate using a vacuum rotary evaporator. The remaining aqueous portion was then extracted three times with dichloromethane (10 ml for each time). The organic layer was dehydrated over anhydrous sodium sulfate and its volume reduced to 1–2 ml with evaporator. The concentrated dichloromethane extracts were transferred to a glass column (1.0 cm i.d., 20 cm length) packed with a mixture of Florisil (80–120 mesh), acidic aluminum oxide and activated carbon (5:5:1 by weight), and followed by a rinse with a mixing solvent (30 ml) of methanol and ethyl acetate (40:60 by volume). The eluate was evaporated to dryness on a rotary evaporator, and the residue was re-dissolved in methanol (5 ml) for estimation by HPLC.

1.6 HPLC-DAD analysis

An Agilent 1100 model high performance liquid chromatography (HPLC), equipped with diode array detector (DAD), was operated to determine the residue of imazaquin. A YWG-C_{18} reversed phase column (250 mm × 4.6 mm i.d., 5-μm porosity) was used and thermostated at 30°C. The mobile phase used was a 65:35 mixture of methanol/water (pH=4) at a flow rate of 0.8 ml/min. The detector was set at 210 nm, and the injection volume was 20 μl. Under these conditions the retention time of imazaquin was about 15.8 min, and the detection limit for imazaquin in soil was 0.015 μg/g.

1.7 Recovery study and statistical analysis

A recovery study was carried out by spiking unamended and BM or CM-amended soil (at the 10% amendment rate by w/w) with imazaquin stock solution to obtain a series of concentrations (0.1, 1, 5 and 10 μg/g). The average recoveries for unamended treatment ranged from 91.2% to 98.4%, and the relative standard deviations (RSD) from 1.3% to 7.6%. Similarly for CM- or BM-amended treatments, it was in the range of 82.3% to 93.6%, and RSD from 4.9% to 9.2%, respectively. As a result, the adopted method could meet the requirement for residual analysis of imazaquin in the investigated soil (data not shown).

Means and standard errors of these 3 replicates for each treatment were shown in the subsequent figures or tables. Analysis of variance (ANOVA) and Duncans Multiple Range test were used to determine significant differences (at $p<0.05$) among each treatment and within groups of treatments in SAS (SAS Institute, Cary, NC, USA).

1.8 Extraction, isolation and identification of the metabolite

On the basis of imazaquin half-lives in unamended and BM-amended soil, we selected the samples at 5, 10, and 30 DAT for metabolite study. The preceding method described in Section 1.5 was used to recover the metabolite. The extraction was dried by rotary evaporation, the residue was re-dissolved in methanol, then separated and purified by preparative thin-layer chromatography (TLC) on precoated 0.25 mm, 20 cm × 20 cm silica gel 60 F_{254} (Merck, Germany). The plates were developed with chloroform-methanol (65:35 by volume). The metabolites were visualized by UV light absorption. The bands on preparative TLC plates were scraped off and extracted with dichloromethane. The suspension was then filtered through a medium-porosity glass filter to yield a filtrate which was concentrated and dried by nitrogen flow to give pure compound proved by no occurrence of other material peaks in HPLC profiles. The metabolites were subjected to IR, MS, and NMR analyses. ^{1}H NMR analysis spectra of the metabolites were recorded using Varian Mercury Plus 400 instrument with $CDCl_3$ as solvents. Chemical shifts were given in parts per million units relative 0.00 in tetramethylsilane (TMS) as an internal standard. For IR analysis, the absorption spectra of metabolites were measured in KBr pellets using a Nicolet AVATAR-360 model FTIR spectrophotometer. For HPLC-ESI-MS analysis, analysis of imazaquin metabolites was performed using an Agilent 1100 series HPLC-MS (Agilent technologies, USA). HPLC was equipped with a 2.1-mm (i.d.) × 150 mm column packed with YWG-C_{18} reversed phase material. A mixture of methanol and water (85:15 by volume) was used as mobile phase at a constant flow rate of 0.2 ml/min. MS was equipped with a quadrupole analyzer and electrospraying ionization (ESI) source operating in positive mode. Ionization conditions of ESI analysis were as follows: dry gas follow 14 L/min; nebulizer pressure 220 kPa; dry gas temperature 300°C; capillary voltage 3500 V; vaporizer temperature 300°C.

2 Results and discussion

2.1 Effect of farm litter on degradation

The data fitting results using the first-order kinetics equation showed that correlation coefficients (r) ranged from 0.9790 to 0.9961 between imazaquin residues and time. As a result, the first-order kinetics was considered to be acceptable, and was applied to all the datasets to facilitate the comparison of rate constants. As for the unamended treatment, the half-life of imazaquin was 21.5 d (Table 3), which was in general agreement with that of the previous reports (7–133 d) (Schroeder, 1993). However, the degradation trend was substantially increased by addition of either farm litters. For BM-amended treatment, the amount of imazaquin left at 30 DAT was only 1.29 mg/kg showing 86.4% loss of imazaquin, while at the same time only 60.5% disappearance occurred for unamended treatment (control). Almost complete degradation (97.8%)

Table 3 Degradation kinetics parameters of imazaquin in the sandy loam soil

Treatment method	$k \pm$ SD ($\times 10^{-2}$ d^{-1})	$t_{1/2} \pm$ SD (d)	r
Unamended (control)	3.22±0.19	21.5±1.6 a	0.9790
CM-amended	4.87±0.47	14.2 ±1.9 b	0.9961
BM-amended	7.66±0.92	9.0 ±0.7 c	0.9932

Incubation temperture: 30°C; application rate: 10% by w/w; moisture level: 60% of WHC_{max}; different lower cases denote the significant difference at $p<0.05$; ±SD (standard deviation, n=3), the data in the following tables (4, 5 and 6) have the same replicates unless otherwise stated.

occurred at 50 DAT (Fig.1), and the estimated half-life of imazaquin was 9.0 d in BM-amended soil, which was almost 2.4 times faster than that in unamended soil. In contrast, the incorporation of CM into soil approximately enhanced imazaquin degradation 1.5 times, and the estimated half-life was 14.2 d. The mechanism for the effect of litter addition on pesticide degradation is very complicated. Addition of organic amendment introduces new kinds of microorganisms and large amount of nutrient elements such as organic carbon, nitrogen and so on. The introduced microorganisms possibly degrade pesticide and also promote the occurrence of co-metabolic biodegradation between the introduced and indigenous microorganisms in soil. Additionally, the nutrient elements can stimulate the increasing proliferation of the indigenous microbes (Barriuso *et al*., 1997). As a result, the combing action of the introduced microbes and nutrient elements in amendments lead to enhanced degradation of pesticides (Ferreira *et al*., 2002). On the other hand, the soil amendment with organic matter can increase magnitude of pesticide sorption, which may lower pesticide degradation and mobility. Because imazaquin was weakly adsorbed to soil (Basham *et al*., 1987), its enhanced degradation in BM- or CM-amended soil was a combing result of the introduced microbes and nutrient elements. Bacterial density in BM was approximately 75.3×10^6 CFU/g, which was about 4.3 times higher than that in CM. In addition, the fungi density was about 1.5 times higher in BM than in CM (Table 2). Higher microbial density possibly introduced more imazaquin-degraders. As a result, imazaquin degradation was about 1.6 times faster in BM-amended soil than in CM-amended soil. Dungan *et al*. (2001) also observed the similar phenomenon that 1,3-dichloropropene degradation was 2.3 and 3.3 times faster, respectively, in soil amended by composted steer and chicken manure than in unamended soil. The biodegradation of atrazine was also enhanced in a loamy soil using farm litter (Gupta and Baummer, 1996) or organic amendments (Houot *et al*., 1998). Since

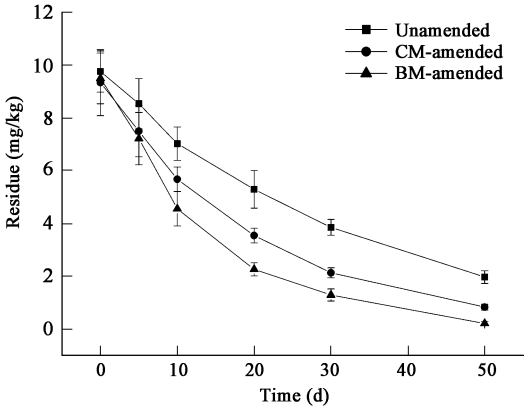


Fig. 1 Effect of BM or CM on imazaquin degradation. Each point represents the mean of triplicate and error bar indicates standard deviation of triplicate (n=3).

BM was found to substantially increase the degradation of imazaquin over that of CM, it was selected for further experiments.

2.2 Effect of temperature on degradation

For the unamended treatments (control), the rate constants (k) at 20, 30 and 40°C were 0.0225, 0.0322 and 0.0439 d^{-1}, and the corresponding half-lives were 30.8, 21.5 and 15.8 d, respectively (Table 4). As the temperature increased from 20 to 40°C, imazaquin degradation approximately increased 2.0 times. The k value approximately increased by a factor of about 1.4 for each 10°C change in temperature. However, for BM-amended treatments, the half-lives of imazaquin varied from 6.7 to 15.4 d over the temperature range of 20 to 40°C, and the k value increased by a factor of about 1.5 for each 10°C change in temperature. The similarly increasing trend was observed for each 10°C change in temperature in both unamended and BM-amended soil. This result suggested that the microorganisms in the investigated soil and BM had the similar sensitivity to temperature. The degradation of pesticide in soil is a result of biological and chemical transformation. Because this experiment was carried out in the dark, photolysis could be negligible and thus chemical process was only involved in hydrolysis. Barkani *et al*. (2005) have reported that imazaquin breaks down very slowly by hydrolysis at pH 3–9 aqueous solution and temperature has slight effect on imazaquin hydrolysis. Therefore, in this research, the increasing degradation of imazaquin with the increase of temperature (20–40°C) possibly resulted from the enhanced activities of microorganisms in soil and BM.

The relationship between temperature and k value close-

Table 4 Effect of temperature on imazaquin degradation in BM-amended soil

Treatment	Incubation temperature (°C)	$k \pm$ SD ($\times 10^{-2}$ d^{-1})	$t_{1/2} \pm$ SD (d)	r
Control	20	2.25±0.14	30.8±3.6 a	0.9812
(unamended)	30	3.22±0.66	21.5±2.8 b	0.9790
	40	4.39±0.54	15.8±2.9 c	0.9456
BM-amended	20	4.50±0.76	15.4±3.6 c	0.9988
	30	7.66±0.67	9.0±1.7 d	0.9932
	40	10.34±2.76	6.7±0.8 e	0.9894

Application rate: 10% by w/w; moisture level: 60% of WHC_{max}; different lower cases denote the significant difference at $p<0.05$.

ly followed Arrhenius equation ($r > 0.95$). The activation energy (E_a) at different temperatures was calculated to be the slope of the plot of ln k vs. $1/T$. The calculated E_a values for unamended and BM-amened treatments were 26.0 and 25.7 kJ/mol, respectively. The sensitivity of the rate of a reaction to change in temperature depends on the value of E_a. Low E_a values are often associated with insensitivity to temperature (Yates and Gan, 1998). In unamended and 10% BM-amended soil, the average increases in degradation rates for each 10°C change were about 1.5 and 1.4-fold, which were lower than the general rule that the rate of a reaction doubles with every 10°C rise in temperature.

2.3 Effect of amendment rate on degradation

The k values increased with the increase of amendment rates of BM except for 15% rate treatment (Table 5). Over the amendment rate range of 5% to 15%, the half-lives of imazaquin decreased by 43.2%. However, no significant difference was observed between 10% and 15% rate treatments, which indicated that higher mix ratio did not necessarily increase imazaquin degradation. The values of k differ significantly between 5% and 10% rate treatments (Table 5), which suggested that the optimal active degradation of imazaquin lied in mix ratio of 10:1 of the soil to BM (Fig.2). The previous conclusion was in general agreement with Namkoong's observation (2002) that addition of organic amendment could increase the degradation of target contaminant, but might inhibit the degradation when an excessive amount of organic amendment was added. The carbon source in the litter must not represent a preferential carbon source that preempts degradation of the target contaminant (Lagrega *et al*., 1994). That is to say, when the added carbon source is preferentially degraded over the target compounds, microbial activity for degrading the target contaminant may be inhibited. In this investigation, BM added as carbon source did not act as competing energy source, and therefore led to an increase of imazaquin degradation.

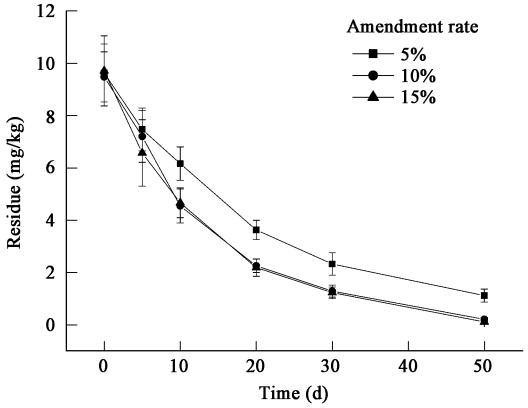


Fig. 2 Effect of amendment rate on imazaquin degradation in BM-amended soil.

Table 5 The first-order kinetics parameters of imazaquin in BM-amended soil at different amendment rates

Amendment rate (%)	$k \pm$ SD ($\times 10^{-2}$ (d^{-1}))	$t_{1/2} \pm$ SD (d)	r
0 (control)	3.22±1.46	21.5±3.2a	0.9790
5	4.28±0.76	16.2±1.8b	0.9942
10	7.66±1.12	9.0±2.5c	0.9932
15	7.53±1.44	9.2±1.9c	0.9766

Incubation temperature: 30°C; moisture level: 60% of WHC_{max}. Different lower cases denote the significant difference at $p<0.05$.

2.4 Effect of moisture level on degradation

The half-lives of imazaquin at 30%, 60% and 80% of WHC_{max} were 9.4, 9.3 and 9.7 d, respectively (Table 6). No significant differences were observed among three moisture treatments, which suggested that the moisture level had negligible effect on imazaquin degradation. Soil water has two roles in pesticide degradation. One is promoting hydrolysis of pesticide, while the other is competing with pesticide for binding sites in soil (Basham *et al*., 1987). Imazaquin breaks down very slowly by hydrolysis, and has a hydrolytic half-life of 5.5 months at pH 9 aqueous solution (Barkani *et al*., 2005). In addition, it is weakly adsorbed to soil (Basham *et al*., 1987). Therefore, the different moisture level in soil had negligible influence on imazaquin degradation.

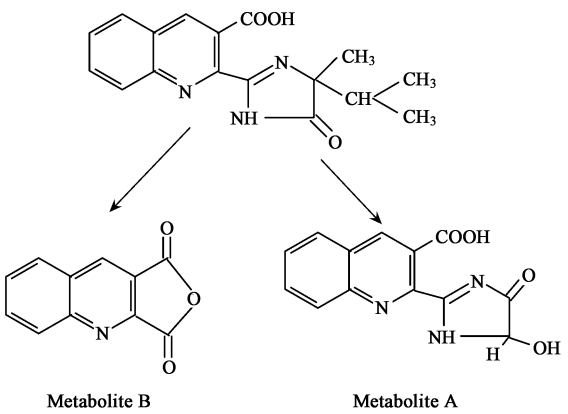
Table 6 First-order kinetics parameters of imazaquin in BM-amended soil at different moisture levels

Moisture level	$k \pm$ SD ($\times 10^{-2}$ d^{-1})	$t_{1/2} \pm$ SD (d)	r
80% WHC_{max}	7.14±1.7	9.7±2.6 a	0.9590
30% WHC_{max}	7.37±1.3	9.4±1.8 a	0.9898
60% WHC_{max}	7.45±1.9	9.3±1.4 a	0.9932

Different lower cases denote the significant difference at $p<0.05$; incubation temperature: 30°C; amendment rate: 10% by w/w.

2.5 Intermediary metabolite formation

According to EPA's registration bulletin (1986), imazaquin readily breaks down via microbial breakdown in soil. It is decarboxylated slowly to CO_2, as well as degraded to the major metabolite CL 266,066 and at least six minor metabolites (U.S. Environmental Protection Agency Bulletin, 1986). However, in this study, only two metabolites at RT 8.4 and 12.9 min were found by comparing the HPLC result of BM-amended treatment with the corresponding control, and they were designated as metabolite A and B, respectively. The highest peak areas of metabolite A and B were both found at 10 d of incubation, and then decreased approximately by 42% and 65%, respectively, at 30 DAT. Metabolite A was identified as 2-(4-hydroxyl-5-oxo-2-imidazolin-2-yl) quinoline acid, based on the following evidence: IR, 3465 (imidazolin-4-OH), 3275 (NH), 3083, 1665 (COOH), 1634 (imidazolin-5-CO); ^{1}H NMR, 8.45 (s, 1H, NH), 5.79 (d, 1H, imidazolin-4-H), 7.41–8.15 (m, 4H, quinoline-5,6,7,8-H), 8.86 (s, 1H, quinoline-4-H), 11.19 (–COOH), 9.79 (–OH); MS, *m/z* 272.1 (M+1), fragment ions 228 (–COOH), 212 (–COOH, –OH), 101 (-nicotinic acid). Metabolite B was identified as quinoline-2,3-dicarboxylic anhydride, based on the following evidence: IR, 1835, 1728 (–COOCO–); ^{1}H NMR, 7.49–8.18 (m, 4H, quinoline-5,6,7,8-H), 8.89 (s,



Metabolite B Metabolite A

Fig. 3 Proposed intermediary metabolites of imazaquin in the sandy loam soil.

1H, quinoline-4-H); MS, *m/z* 200.4 (M+1). The proposed structures of two intermediary metabolites are shown in Fig.3. As can be inferred from the structure, metabolite A, bearing imidazolinone ring, was formed accompanied by the loss of isopropyl group and hydroxylation at the 4-position of imidazolinone ring. In contrast, metabolite B resulted from the detachment of imidazolinone ring and the forming of dicarboxylic anhydride. It was coincident with Barkani's report (2005) that metabolite B was found in investigation on phototransformation of imazaquin. This author also detected one imazapyr photoproduct, pyridine-2,3-dicarboxylic anhydride, on soil surface (Wang *et al*., 2004), which has the similar forming mechanism with metabolite B. Accordingly, for imidazolinone family herbicide the dicarboxylic anhydride can occur under different environmental conditions.

The observations in the present study indicated that both BM and CM could significantly increase imazaquin degradation in the sandy loam soil. Consequently, not only can the application of BM or CM increase the soil fertility, but also contribute to promote the degradation of imazaquin residues in legume crops, which will be more safe to the following susceptible crops.

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