Postprint of: Pest Management Science (2019) Article in Press

**A clay-based formulation of the herbicide imazaquin containing exclusively the
biologically active enantiomer**

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Running Title: Clay-based formulation of the biologically active imazaquin enantiomer

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Abstract

BACKGROUND: Imazaquin is a chiral herbicide which displays high mobility in soils. Like other imidazolinones, imazaquin is available for use only as racemic mixture of its enantiomers. In this work, several clay materials were assayed as adsorbents of imazaquin, and then the most suitable material was selected to prepare a clay-based slow release imazaquin nanoformulation containing exclusively the biologically active *R*-enantiomer. Next, laboratory experiments were conducted to illustrate the benefits of using the clay-based *R*-imazaquin formulation over the free (non-supported) racemic herbicide or the free pure *R*-imazaquin enantiomer regarding its leaching behavior and bioefficacy.

RESULTS: The clay material selected as a carrier for *R*-imazaquin, hexadecyltrimethylammonium-saturated montmorillonite (SA-HDTMA), combined a high affinity for the herbicide and a high stability of the clay-herbicide adsorption complex. In a simulated scenario of high water input shortly after herbicide application, the clay-based *R*-imazaquin formulation displayed reduced leaching and increased bioefficacy compared to free racemic imazaquin and free *R*-imazaquin.

CONCLUSION: The new clay-*R*-imazaquin formulation prepared, besides avoiding the environmental impact caused by the application of the less active *S*-enantiomer, reduced the herbicide leaching losses and prolonged the herbicidal activity, by increasing the residence time of the herbicide in the topsoil.

Keywords: bioefficacy; chiral pesticides; clays; imidazolinones; leaching; nanoformulation

1 INTRODUCTION

Imazaquin [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)quinoline-3-carboxylic acid] is a herbicide belonging to the group of imidazolinones that is applied either pre- or post-emergence for the control of weeds mainly in ornamentals, turf, and soybean crop. Imidazolinones act by inhibition of the enzyme acetohydroxyacid synthase, which catalyzes reactions in the biosynthetic pathway of branched chain amino acids, leucine, isoleucine, and valine.¹

Imazaquin is a chiral compound due to the presence of an asymmetrical carbon in its structure. This implies that it can exist as two enantiomers: *S*-imazaquin and *R*-imazaquin (Fig. 1). It has been reported that the herbicidal activity of imidazolinones mainly is associated with the *R*-enantiomers.^{2,3} Nonetheless, formulations of imazaquin enriched with the active enantiomer are not currently commercialized; imazaquin is marketed exclusively as racemic mixture. Several factors support the use of pure *R*-imazaquin formulations: (i) the herbicidal activity of the *R*-enantiomer has been reported to be nearly eight-fold greater than that of the *S*-enantiomer,^{2,3} (ii) both enantiomers of imazaquin are quite persistent in soil,³⁻⁵ and (iii) interconversion between the enantiomers does not appear to occur in soil.⁵

One of the most relevant soil properties that can affect the imazaquin behavior is pH, since this herbicide is an amphoteric compound with several functional groups with acid-base character. At common pH levels of agricultural soils (pH > 5), the anionic form of imazaquin predominates,^{6,7}. This leads to weak adsorption and, as a result, to a high mobility of imazaquin in soils,⁸⁻¹⁰ which may cause groundwater or surface water pollution, as well as a decrease of its efficacy, as the herbicide may be easily displaced from the topsoil. This behavior may require higher rates of imazaquin application to compensate for the transport losses, leading to an increase in costs and pollution.

A possible solution to reduce pesticide transport losses is the use of controlled release formulations from where the pesticide is delivered slowly over time.¹¹ Several materials have been proposed as carriers of imidazolinone herbicides, such as diethylaminoethyl cellulose, Dowex, polyethylenimine-cellulose, and smectites modified with polycation polymers.¹²⁻¹⁴ For the

specific case of imazaquin, several controlled release formulations based on clays and organoclays have been designed. Polubesova et al.¹⁵ showed that an organoclay-imazaquin formulation prepared from a crystal violet-montmorillonite complex reduced imazaquin leaching in soil. Undabeytia et al.¹⁶ observed a reduction in imazaquin release of approximately 50% by the use of Fe-pillared clay mineral formulations prepared in the presence of cyclodextrin, compared to the use of a standard commercial formulation. However, no attempts have been made to prepare imazaquin formulations containing exclusively the biologically-active (*R*) enantiomer, which would have the additional value of avoiding the introduction of the non-active enantiomer into the environment.

The main objectives of this work were: (i) to assess the potential of different clays and organoclays as adsorbents of imazaquin enantiomers, (ii) to prepare a clay-imazaquin formulation, from the most suitable clay material, containing exclusively the biologically active *R*-enantiomer, and (iii) to evaluate, under laboratory conditions, the benefits of applying the clay-based *R*-imazaquin formulation to an agricultural soil on the basis of its leaching behavior and bioefficacy under a simulated scenario of intensive rain.

2 MATERIALS AND METHODS

2.1 Herbicide

Analytical standard-grade racemic imazaquin (purity > 99.9%) was supplied by Sigma-Aldrich (Spain). The pure *R*-enantiomer of imazaquin was isolated by semipreparative high performance liquid chromatography (HPLC) from a 500 mg L⁻¹ *rac*-imazaquin solution prepared in 0.001 M HCl+acetonitrile (6+4 by volume), following the procedure described in López-Cabeza et al.⁵ Briefly, following injection of 50 µL of the *rac*-imazaquin solution into the HPLC system, the peak eluting at a retention time of 7.3 min was collected, and then neutralized with 0.1 M NaOH to a pH value close to 6. Afterwards, the acetonitrile was evaporated from the neutralized collected fraction by means of a soft stream of nitrogen. The concentration of the resulting *R*-imazaquin aqueous solution was ~30 mg L⁻¹ with an enantiomeric purity > 99%. The *R*-imazaquin isolation

described above was performed using the same HPLC equipment and chromatographic conditions as those detailed in section 2.8 for the analysis of the herbicide, but using 0.001 M HCl+acetonitrile (6+4 by volume) as mobile phase instead of 0.01 H₃PO₄+acetonitrile (6+4 by volume) to avoid the presence of phosphate in the final *R*-imazaquin solution.

2.2 Clay materials and soil

The clay materials tested as imazaquin adsorbents were carbonated hydrotalcite (HT-CO₃), calcined hydrotalcite (HT₅₀₀), elaidate-saturated hydrotalcite (HT-ELA), Arizona montmorillonite (SAz-1), and hexadecyltrimethylammonium-saturated Arizona montmorillonite (SA-HDTMA). These adsorbents had previously been characterized in depth and were selected for being representative of unmodified and organically-modified cationic and anionic clay minerals.¹⁷⁻¹⁹ The HT-CO₃ sample was synthesized by the conventional coprecipitation method.^{17,20} A portion of this HT-CO₃ was calcined at 500 °C for 4 h to obtain the HT₅₀₀ sample. The organo-hydrotalcite (HT-ELA) was prepared according to the reconstruction method by re-hydration of a sample of HT₅₀₀ with an alkaline aqueous elaidate solution, as described in Celis et al.¹⁷ The organo-montmorillonite SA-HDTMA was prepared by modification of SAz-1 Ca-rich Arizona montmorillonite from the Source Clay Repository of The Clay Minerals Society (Purdue University, West Lafayette, IN) with HDTMA cations by an exchange reaction, as detailed in Gámiz et al.¹⁸ Some characteristics of the unmodified and modified clay samples used in this study are given in Table 1.

An agricultural soil from Seville (SW Spain) was collected (0-20 cm), air-dried, sieved (2 mm), and stored at 4 °C prior to use. It was a sandy loam soil with pH = 8.3, 20% clay, 6% silt, 74% sand, and 0.50% organic C.

2.3 Adsorption experiments

The adsorptive capacity of the clays and organoclays was evaluated by batch adsorption experiments. For this purpose, triplicates of each clay material (HT-CO₃, HT₅₀₀, HT-ELA, SAz-1, or

SA-HDTMA) (10 mg) were equilibrated with a 1 mg L⁻¹ aqueous solution of *rac*-imazaquin (8 mL) by shaking for 24 h in an end-over-end shaker at 20 ± 2 °C. Next, the suspensions were centrifuged (8000 rpm, 15 min) and the supernatants were filtered using GHP membrane disk filters (0.45 µm) to determine the solution concentration of each imazaquin enantiomer (C_e) by chiral HPLC. The amount of *S*- and *R*-imazaquin adsorbed (C_s) was calculated from the difference between the initial (C_{ini}) and equilibrium (C_e) solution concentration of each enantiomer. The percentage of enantiomer adsorbed (%Ads) was calculated according to: %Ads = [(C_{ini} - C_e)/C_{ini}]×100, and distribution coefficients, K_d (L kg⁻¹), as K_d = C_s/C_e. Triplicate tubes with the initial imazaquin solution without adsorbent were also shaken for 24 h as controls and showed no losses of herbicide due to adsorption to the tubes or to the filter material.

2.4 Preparation and stability study of clay-*R*-imazaquin formulations

Clay-*R*-imazaquin formulations (HT-*R*-imazaquin and SA-HDTMA-*R*-imazaquin) were prepared by mixing a portion of HT₅₀₀ (15 mg) or SA-HDTMA (25 mg) with a 30 mg L⁻¹ aqueous solution of *R*-imazaquin (10 mL). The suspensions were shaken for 24 h (20 ± 2 °C) and then vacuum-filtered. The solids (clay-*R*-imazaquin formulations) were dried at 60 °C, homogenized in an agate mortar, and stored at room temperature and darkness. The filtrates were analyzed by chiral HPLC to determine indirectly the amount of *R*-imazaquin retained in the formulations. In addition, the amount of *R*-imazaquin in the formulations was also determined by direct extraction, following procedures based on those previously described for similar clay-pesticide systems.^{21,22} In the case of the HT-*R*-imazaquin, an aliquot of the formulation (1 mg) was treated in triplicate with HCl (37%, 1 mL) and then shaken for a few minutes until the complete dissolution of the hydrotalcite layers. Next, a mixture of H₃PO₄ (pH = 2)+acetonitrile (6+4 by volume) (9 mL) was added, and the resulting solution was analyzed by chiral HPLC. For SA-HDTMA-*R*-imazaquin, the amount of *R*-enantiomer was determined by extracting in triplicate of a portion of formulation (1 mg) with a mixture of H₃PO₄ (pH = 2)+acetonitrile (6+4 by volume) (10 mL). After 24 h of shaking, the suspension was centrifuged and the supernatant solution filtered and analyzed by chiral HPLC.

The recovery of the extraction procedures was confirmed by comparing the amount of *R*-imazaquin in the formulations obtained in these direct extractions with that determined indirectly from the analysis of the filtrates. The value of this recovery was > 85%. In order to check the stability of both formulations over time, the amount of *R*-imazaquin adsorbed on the clay materials was also extracted and analyzed as described above 1 month after the preparation of the formulations.

2.5 Detailed study of the degradation of the herbicide in the HT-imazaquin formulation

A detailed extraction kinetic study was carried out to get additional information about the causes of the instability observed for the HT-imazaquin complex. This study was conducted using an HT-supported *rac*-imazaquin complex prepared in the same manner as described in section 2.4, but using racemic imazaquin instead of the pure *R*-enantiomer. The rationale for using racemic imazaquin was its availability and that, in an achiral environment, the chemical degradation of both enantiomers of imazaquin was expected to be identical. Periodically (0, 2, 6, 9, 15, 24, 34 and 44 days after preparation), a sample of the HT-*rac*-imazaquin formulation (1 mg) was extracted in triplicate following the procedure detailed in section 2.4 and the extract was analyzed by achiral HPLC. The resulting concentrations were fitted to first-order kinetics using Sigma Plot 12.5 software. A 30 mg L⁻¹ *rac*-imazaquin aqueous solution was also prepared and used as a control. The pH of this imazaquin control solution was adjusted to a value similar to that of the filtrate obtained during the preparation of the HT-based formulation (pH = 10).

The degradation product of imazaquin was characterized by liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). For this aim, a portion of formulation (1 mg) stored for 54 days after its preparation was dissolved in HCl 37% (1 mL) and diluted with a solution of 0.1% formic acid in Milli-Q water (1:1000). An aliquot of the resulting extract (0.5 mL) was analyzed by HPLC-MS/MS. The sample was injected into the system by an autosampler (HTS PAL, CTC Analytics) equipped with two six-port valves for column switching, a sample loop, and an on-line extraction cartridge (two stacked Phenomenex Gemini-NX C18 cartridge precolumns, 4 mm

length $\times$ 3 mm internal diameter and 5 μ m particle size). The chromatography system consisted of a pump used solely for samples loading (Jasco PU-980) and a binary gradient HPLC pump (Agilent 110 series) for delivery of the mobile phase. The HPLC column was connected to an API 4000 triple quadrupole mass spectrometer equipped with a turbo ion spray (TIS) source operating in positive mode. The LC conditions were as follows: Phenomenex Aqua 125 A C18 chromatographic column of 50 mm length $\times$ 2 mm internal diameter and 5 μ m particle size. The mobile phase consisted of 0.1% formic acid in water (eluent A) and acetonitrile (eluent B). Initially, the column was saturated with 100% eluent A. Compounds were eluted with a linear gradient from 0 to 100% acetonitrile within 10 min, followed by an isocratic phase of 2 min. Initial conditions were reestablished within 0.1 min, and then the column was equilibrated with eluent A for 2 min prior to the next analysis. The flow of mobile phase was 0.2 mL min⁻¹ during all the analyses.

2.6 Column leaching experiment

The soil leaching experiment was performed in triplicate using glass columns (30 cm length $\times$ 3.1 cm internal diameter). Glass wool and sea sand (10 g) were placed on the bottom of the columns to prevent loss of soil particles, and then the columns were hand-packed with soil (160 g). Finally, sea sand (10 g) was added on the top of the columns to allow a uniform distribution of the herbicide and water added on the soil surface. The soil columns were saturated by adding deionized water (100 mL) and allowed to drain for 24 h. The pore volume of the soil columns was calculated as the amount of water retained by the column after saturation and 24-h drainage ($V_p = 61 \pm 1$ mL). Next, *rac*-imazaquin at a rate of 200 g ha⁻¹ (2.5 mL of a 6 mg L⁻¹ aqueous solution) or *R*-imazaquin at a rate of 100 g ha⁻¹ (2.5 mL of a 3 mg L⁻¹ aqueous solution or 1 mg of SA-HDTMA-*R*-imazaquin formulation with a *R*-imazaquin content of 8 mg g⁻¹) were applied in triplicate on the column surface. Daily, deionized water (15 mL) was added to the columns, and after allowing to drain for 24 h, the leachates were collected, filtered, and analyzed by chiral HPLC to determine the concentration of the imazaquin enantiomers.

2.7 Bioassay

A bioassay was performed to assess the herbicidal activity of imazaquin following a simulated intensive rain event just after application of the herbicide either as racemic mixture of enantiomers or as pure *R*-enantiomer, the latter applied free (water-dissolved) or supported on SA-HDTMA. Triplicate 36 cm² surface × 5 cm height pots were filled with soil (100 g). Next, imazaquin was applied as *rac*-imazaquin at a rate of 80 g ha⁻¹ (5 mL of a 6 mg L⁻¹ aqueous solution) or as pure *R*-imazaquin at a rate of 40 g ha⁻¹ (5 mL of a 3 mg L⁻¹ aqueous solution or 1.8 mg of SA-HDTMA-based formulation with a herbicide content of 8 mg g⁻¹). Immediately after application of the herbicide, deionized water (150 mL) was slowly added at once to each pot using a beaker, to simulate a rain event of 40 L m⁻². The pots were allowed to drain for 24 h and the leachates were collected and analyzed by chiral HPLC. Triplicate control pots of soil without herbicide were also prepared. Then, 12 seeds of *Brassica oleracea botrytis* (Vilmorin, France) were sown in each pot and these were moistened daily with deionized water. Earlier experiments had shown that this plant was particularly susceptible to imidazolinones.^{12,23} After 2 weeks, plants were harvested from the pots, the length of their stems and roots were measured and, finally, their aerial parts were cut and weighed to determine the aerial biomass.

2.8. Analysis of imazaquin enantiomers

The analysis of imazaquin was conducted by reversed-phase achiral or chiral HPLC using a Waters 600E chromatograph coupled to a Waters 996 PDA detector. The chromatographic conditions for both HPLC modalities were: 25 µL injection volume, 0.01 M H₃PO₄ aqueous solution (pH = 2)+acetonitrile (6+4 by volume) isocratic mixture at a flow rate of 1 mL min⁻¹, and UV detection wavelength at 250 nm. For the achiral analysis, a Kinetex C18 chromatographic column of 150 mm length × 4.6 mm internal diameter and 5 µm particle size (Phenomenex) was used. The retention time for *rac* (*S+R*)-imazaquin was 3.3 min. For the chiral analysis, the column was a Chiralcel OD-3R analytical column of 150 mm length × 4.6 mm internal diameter and 3 µm

particle size (Chiral Technologies Europe, France). Under these conditions, *S*-imazaquin eluted at 6.4 min and *R*-imazaquin eluted at 7.3 min.⁵ Both for achiral and chiral analysis, five external standard solutions of analytical standard-grade *rac*-imazaquin with concentrations in the range 0.1-6 mg L⁻¹ were also injected. Instrumental limit of quantification, defined as the concentration resulting in a signal to noise ratio of 10:1,²⁴ was 0.02 mg L⁻¹ for *S*-, *R*-, and *rac*-imazaquin.

2.9 Statistics

Statistical analysis was carried out using SigmaPlot 12.5. The results were expressed as mean ± standard error to indicate variability among triplicates. Leaching and bioassay data were analyzed using one-way ANOVA followed by Fisher's LSD tests to establish if statistically significant differences existed between treatments. For all statistical analyses, a P value of 0.05 was set as the level of significance.

3 RESULTS AND DISCUSSION

3.1 Adsorption of imazaquin enantiomers on the clay materials and stability of the adsorption complexes

Imazaquin enantiomers did not adsorb on either the carbonated hydrotalcite (HT-CO₃) or the elaidate-modified hydrotalcite (HT-ELA) sample (%Ads < 1%). The lack of imazaquin adsorption on the HT-CO₃ sample can be attributed to the high affinity of carbonate for the hydroxide layers of HT, which prevented this anion from being displaced by the herbicide.^{17,25,26} In the case of HT-ELA, the null adsorption observed can be attributed to repulsion between the anionic form of imazaquin at the pH value of the suspensions (pH > 6) and the elaidate anions present at the surfaces of HT-ELA, as Z-potential measurements have indicated that, upon saturation with ELA, the HT surface changes from positively charged to negatively charged.¹⁷ In contrast, the adsorption of imazaquin enantiomers on HT₅₀₀ was close to 100% (Table S1). This strong adsorption was due to the well-known ability of calcined hydrotalcites to adsorb anionic species

from aqueous solution during rehydration and reconstruction of the original layered structure of HT.²⁵⁻²⁹

In the case of the montmorillonitic clays, the original SAz-1 sample did not adsorb imazaquin (%Ads < 1%). Undabeytia et al.¹⁶ attributed the lack of adsorption of imazaquin on montmorillonite to be due to electrostatic repulsion between the negatively charged clay mineral layers and imazaquin anions. However, for the HDTMA-modified montmorillonite sample (SA-HDTMA) the adsorption of imazaquin enantiomers was very high (> 95%, Table S1). The incorporation of HDTMA cations into the interlayer of montmorillonite changed the nature of the surface from hydrophilic to hydrophobic, allowing hydrophobic interactions between the hydrocarbon chain of HDTMA and the quinolone group of imazaquin and electrostatic interaction between the anionic herbicide and the positively charged quaternary ammonium groups of HDTMA.^{18,30}

On the basis of the adsorption results, we concluded that both HT₅₀₀ and SA-HDTMA could be suitable carriers for the development of slow release formulations of the active enantiomer of imazaquin. Therefore, HT-*R*-imazaquin and SA-HDTMA-*R*-imazaquin complexes were prepared and their storage stability tested. The quantity of imazaquin extracted from the formulation SA-HDTMA-*R*-imazaquin (8 mg g⁻¹) remained fairly stable after 1 month. However, in the case of HT-*R*-imazaquin, the amount of herbicide initially present in the formulation (10 mg g⁻¹) was reduced by 87 % after 1 month from its preparation (Fig. S1 in the supplementary material). It was concluded that while HT₅₀₀ could be useful for the removal of imazaquin from aqueous solution, a poor stability of the adsorption complex might limit its application as a support for this herbicide. In contrast, the high adsorption capacity combined with the high stability of the SA-HDTMA-imazaquin adsorption complex made SA-HDTMA an interesting adsorbent for its further evaluation as a carrier for the slow release of imazaquin.

3.2 Further assessment of the degradation of imazaquin adsorbed to HT₅₀₀

Figure 2 depicts the degradation curve of *rac*-imazaquin supported on hydrotalcite and that of the herbicide in aqueous solution (30 mg L⁻¹) at a pH $\approx$ 10. This pH was chosen because it was similar to that of the filtrate obtained during the preparation of the HT-imazaquin formulation, and thus representative of the alkaline conditions at which imazaquin existed in the HT formulation. The curve of the control experiment shows that degradation of imazaquin in the absence of HT₅₀₀ was negligible under the experimental conditions: 25 °C, darkness and alkaline pH. However, the amount of imazaquin in the HT-imazaquin complex rapidly decreased with time. It is interesting to note that this experiment was repeated with other imidazolinone herbicides (imazapyr, imazethapyr, and imazamox) and the results obtained were very similar (Fig. S2). In all cases, degradation of the herbicide on the hydrotalcite was faster than in the control aqueous solutions, and the rate of degradation was approximately the same for all the imidazolinone herbicides ($t_{1/2}$ = 11-12 days, Table S2). This may indicate that the degradation occurred at the imidazolinone ring, which is a common structural element of all these compounds (Fig. S3).

In the HPLC chromatograms, upon dissipation of imazaquin, a second peak was observed, which increased over time. In order to characterize this suspected degradation product, a portion of the HT-imazaquin formulation was extracted 54 days after its preparation and the extract was analyzed by HPLC-MS/MS, as described in section 2.5. The full scan chromatogram of this extract showed two prominent peaks at retention times of 6.36 and 7.26 min (Fig. 3). The mass spectrum associated with the first peak consisted of signals at m/z values of 285.1, 313.1, 330.1, whereas the mass spectrum associated with the second peak only consisted of one major signal at m/z = 312.1, assigned to parent imazaquin. For further characterization of the first eluting peak (and suspected degradation product), MS/MS spectra of the three precursor ions m/z = 330.1, 313.1 and 285.1 were acquired (Fig. S4). The MS-MS spectrum of the precursor ion m/z = 330.1 showed signals for numerous fragments including those with m/z values of 285.1 and 313.1. The mass spectra are consistent with 2-[1-carbamoyl-1,2-dimethylpropyl]-carbamoyl]-3-quinolinecarboxylic acid (Fig. S5), a known hydrolysis product of imazaquin and also a soil and

plant metabolite.³¹⁻³⁴ This metabolite is very soluble in water and, unlike imazaquin, non-phytotoxic.³² Hydrotalcite-like compounds are well known for their catalytic properties^{35,36} and we thus conclude that the fairly rapid degradation of imazaquin observed when associated with hydrotalcite was due to chemical degradation by solid-phase, hydrolytic imidazolinone ring opening, mediated by HT.

3.3 Use of SA-HDTMA as *R*-imazaquin carrier for the slow release of the herbicide

3.3.1 Column leaching experiment

Fig. 4 shows the relative and cumulative breakthrough curves (BTCs) for imazaquin obtained in the column leaching experiment. The herbicide was added to the columns as: i) free (water-dissolved) racemic (*S+R*) mixture, ii) free (water dissolved) pure *R*-enantiomer, or iii) SA-HDTMA-*R*-imazaquin formulation. The three applications were normalized to achieve the same amount of active enantiomer (*R*-imazaquin) added to the columns. As expected, the maximum concentration of imazaquin ($C_{\max}$) in leachates from the columns treated with pure *R*-enantiomer ($C_{\max} = 0.065\text{--}0.085 \text{ mg L}^{-1}$) was lower ($P < 0.05$) than that from the columns treated with racemic imazaquin ($C_{\max} = 0.193 \text{ mg L}^{-1}$) (Fig. 4a, Table 2). This reflected the lower dose of total imazaquin needed when the herbicide is applied as biologically-active *R*-enantiomer. In addition, relative curves show that, in the columns treated with the clay-based formulation, the maximum concentration of imazaquin appeared after applying a higher amount of water (120 mL or $2 \times V_p$) than for the columns where the herbicide was applied free, either as a racemic mixture or as pure *R*-enantiomer (90 mL or $1.5 \times V_p$) (Table 2). This delay was also apparent in the cumulative BTCs (Fig. 4b), which also revealed that the total amount of imazaquin leached decreased from 86% for the columns where the herbicide was added as aqueous solution to 75% when the clay-formulation was applied ($P < 0.05$). The greater retardation of the herbicide observed in the columns treated with SA-HDTMA-*R*-imazaquin was attributed to a slow release of the herbicide from this clay-based formulation that limited the amount of imazaquin available for leaching. This reduced leaching of imazaquin was expected to result in an increase in its residence time in the

soil surface layer, which should lead to an increase in biological efficacy of the herbicide compared to its application in an immediately available (free) form.^{37,38}

3.3.2 Bioassay

A bioassay was carried out to test the impact of the SA-HDTMA-*R*-imazaquin clay-based formulation on the efficacy of imazaquin under a simulated worst-case scenario of leaching by intensive rain (40 L m⁻²) occurring immediately after herbicide application. Previous studies have addressed the bioefficacy of organoclay-based formulations of acid herbicides without prior leaching, showing their adequate weed control at application doses similar to those recommended for the herbicides formulated in an immediately available form.^{12,39}

In agreement with the results of the column leaching experiment, the analysis of the imazaquin present in the leachates collected from the pots revealed that significantly ($P < 0.05$) less herbicide losses occurred for the pots treated with SA-HDTMA-*R*-imazaquin (29%) compared to the pots treated with the free herbicide, either as racemic mixture or pure *R* enantiomer (> 95%).

The small amount of imazaquin that remained in the pots treated with free (water dissolved) *rac*-imazaquin and *R*-imazaquin was found to be sufficient to significantly ($P < 0.05$) reduce the root length (Fig. 5a), but not the stem length or aerial biomass of the test plant (Fig. 5b and 5c). In contrast, for the pots treated with the SA-HDTMA-*R*-imazaquin formulation, the inhibition of root growth was significantly more pronounced than for the pots treated with free (water-dissolved) herbicide (Fig. 5a) and was accompanied by a significant reduction in stem length and aerial biomass (Fig. 5b and 5c). The pronounced reduction in root growth probably led to limited uptake of nutrients, explaining a subsequent reduction in plant growth, an effect previously described for other herbicides of the imidazolinone group.^{1,40,41} The greater herbicidal effect observed for the application of the SA-HDTMA-*R*-imazaquin formulation can be attributed to an increase in the residual amount of herbicide in the surface soil as a result of its sustained release from the clay-formulation, which prevented extensive leaching due to the simulated intensive rain event. These results are similar to those obtained in an experiment performed by Gámiz et

al.⁴² in which the amendment of pots with a spermine-modified montmorillonite led to a reduction in the leaching losses of the herbicide fluometuron, which increased its efficacy after a simulated heavy rainfall event conducted immediately after herbicide application.

4 CONCLUSIONS

Calcined hydrotalcite (HT₅₀₀) and hexadecyltrimethylammonium-modified Arizona montmorillonite (SA-HDTMA) displayed a great affinity for the herbicide imazaquin. However, HT₅₀₀ was not an optimal carrier for the preparation of imazaquin formulations, due to the instability of the adsorbed imazaquin. This instability was attributed to fast degradation of the herbicide by hydrolytic opening of the imidazolinone ring, which was mediated by the clay, yielding the (non-phytotoxic) hydrolysis product 2-[1-carbamoyl-1,2-dimethylpropyl]-carbamoyl]-3-quinolinecarboxylic acid. No hydrolysis was observed when the herbicide was adsorbed on SA-HDTMA.

The application of the free biologically-active *R*-enantiomer, instead of the free racemic (*S+R*) mixture of imazaquin, reduced the total herbicide residues in soil column leachates, since the application of the non-active *S* enantiomer was avoided. Bioassays demonstrated that, under a simulated worst-case scenario of leaching by intensive rain occurring immediately after herbicide application, the herbicidal activity of the free *R*-enantiomer applied at 40 g ha⁻¹ was small and not significantly different from that of free *rac*-imazaquin applied at 80 g ha⁻¹. In contrast, when *R*-imazaquin was applied as SA-HDTMA-*R*-imazaquin formulation (at 40 g ha⁻¹) herbicide losses by leaching during the simulated rain event were significantly reduced leading to higher residual herbicidal effect compared to the experiments with *R*- or *rac*-imazaquin applied in aqueous solutions. These results indicate that the application of imazaquin as a clay formulation containing exclusively the biologically-active enantiomer, besides the added value of avoiding incorporation of the non-active *S*-enantiomer into the environment, would reduce the high leaching potential of the herbicide and improve its efficacy, particularly in cases of heavy rainfall episodes occurring shortly after herbicide application. These outcomes encourage further

evaluations of organoclays as carriers of biologically-active enantiomers of soil-applied chiral pesticides, which should include experiments under field conditions and, possibly, alternative organic cations as suitable modifiers that ensure the eco-compatibility of the resultant clay-based formulation.

ACKNOWLEDGEMENTS

This work was financed by the Spanish Ministry of Economy and Competitiveness (MINECO grants AGL2014-51897-R and AGL2017-82141-R), co-financed with FEDER-FSE funds. R. López-Cabeza also thanks MINECO for a pre-doctoral fellowship (grant BES-2012-059945) and B. Gámiz and I. Buerge for valuable suggestions.

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FIGURE CAPTIONS

Figure 1. Chemical structures of *S*-imazaquin and *R*-imazaquin.

Figure 2. Degradation curve of imazaquin adsorbed on HT₅₀₀ and imazaquin in aqueous solution (30 mg L⁻¹) at a pH $\approx$ 10. Error bars correspond to standard errors of triplicate measurements.

Figure 3. LC-MS chromatogram (full scan) of the imazaquin-HT₅₀₀ formulation extracted 54 days after its preparation and the MS spectra obtained from the peaks at 6.36 and 7.26 min. Signal intensities were normalized to 100%.

Figure 4. Relative (a) and cumulative (b) breakthrough curves (BTCs) of imazaquin in soil columns upon application of *rac*-imazaquin as free compound, *R*-imazaquin as free compound, and *R*-imazaquin as SA-HDTMA formulation. Error bars correspond to the standard errors of triplicate columns.

Figure 5. Effect of imazaquin on root length (a), stem length (b), and biomass (c) of *Brassica oleracea botrytis* in soil with different treatments: control (no herbicide), free racemic mixture of imazaquin, free *R*-imazaquin, and SA-HDTMA-*R*-imazaquin formulation. Different letters above the bars indicate statistically significant differences between the treatments ($P < 0.05$).

Table 1. Characteristics of the unmodified and modified clay samples used in this study

Sample	Main exchange ion	Mass (%)				OIS [†] (%)	d ₀₀₁ [‡] (Å)
		Mg	Al	C	N		
HT-CO ₃	carbonate	24.0	8.5	2.2	< 0.05	-	7.7
HT ₅₀₀	-	38.6	13.7	0.9	< 0.05	-	-
HT-ELA	elaidate	12.2	4.3	39.1	< 0.05	114	35.5
SAz-1	calcium	n.d. [§]	n.d.	< 0.1	< 0.05	-	15.2
SA-HDTMA	hexadecyltrimethylammonium	n.d.	n.d.	22.14	1.26	101	22.5

[†] Organic ion saturation: percentage of the anion exchange capacity of HT (315 cmol kg⁻¹ clay) or the cation exchange capacity of SAz-1 (120 cmol kg⁻¹ clay) compensated by organic ions (calculated from the C and N content of the samples).

[‡] Basal spacing value.

[§] Not determined.

Table 2. Summary of (*S+R*)- and *R*-imazaquin column leaching data obtained from the breakthrough curves (BTCs) of free *rac*-imazaquin, free *R*-imazaquin, and SA-HDTMA-*R*-imazaquin in the soil

	$C_{\max}^{\dagger}$	Position of $C_{\max}^{\ddagger}$		Total leached [§]
	(mg L ⁻¹)	(mL)	($\times V_p$)	(%)
Rac-(<i>S+R</i>)-imazaquin (free)	0.193 ± 0.001	90	1.5	86 ± 1
Pure <i>R</i> -imazaquin (free)	0.085 ± 0.003	90	1.5	86 ± 0
Pure <i>R</i> -imazaquin (formulation)	0.065 ± 0.007	120	2.0	75 ± 4

[†] $C_{\max}$: maximum concentration of *R*- or (*S+R*)-imazaquin in leachates $\pm$ standard error.

[‡] Position of $C_{\max}$: mL or number of pore volumes (V_p) of water added at which $C_{\max}$ appeared in leachates.

[§] Total amount of *R*- or (*S+R*)-imazaquin recovered in leachates $\pm$ standard error.

Figure 1. Chemical structures of *S*-imazaquin and *R*-imazaquin.

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Supplementary data for

**A clay-based formulation of the herbicide imazaquin containing exclusively the
biologically active enantiomer**

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Number of tables: 2

Number of figures: 5

Table S1. Adsorption percentage (%Ads) and distribution coefficient (K_d) for imazaquin on HT₅₀₀ and SA-HDTMA at a single initial racemic-imazaquin concentration of 1 mg L⁻¹.

		%Ads	K_d (L kg ⁻¹)
HT ₅₀₀	<i>S</i> -imazaquin	100.0 ± 0.0 [†]	> 10000
	<i>R</i> -imazaquin	100.0 ± 0.0	> 10000
	(<i>S+R</i>)-imazaquin	100.0 ± 0.0	> 10000
SA-HDTMA	<i>S</i> -imazaquin	95.6 ± 0.2	9164 ± 586
	<i>R</i> -imazaquin	95.9 ± 0.2	9504 ± 384
	(<i>S+R</i>)-imazaquin	95.8 ± 0.2	9322 ± 459

[†]Value ± standard error

Table S2. First-order degradation parameters for imazaquin, imazapyr, imazethapyr and imazamox supported on HT.

	k (d ⁻¹)	t _½ (d)	R ²
Imazaquin	0.0565 ± 0.0038 [†]	12	0.983
Imazapyr	0.0555 ± 0.0116	12	0.832
Imazethapyr	0.0623 ± 0.0109	11	0.889
Imazamox	0.0609 ± 0.0910	11	0.917

[†]Value ± standard error

Figure S1. Stability of imazaquin adsorbed on SA-HDTMA and HT₅₀₀ over time.

Figure S2. Degradation curves of imazaquin, imazapyr, imazethapyr and imazamox adsorbed on HT₅₀₀ and in control aqueous solution (30 mg L⁻¹) at a pH ≈ 10.

Figure S3. Chemical structure of imazaquin (a), imazapyr (b), imazethapyr (c) and imazamox (d).

Figure S4. MS/MS spectrum of precursor ions with m/z 330.1 (a), 313.1 (b) and 285.1 (c). Signal intensities were normalized to 100%.

Figure S5. Chemical structure of 2-(1-carbamoyl-1,2-dimethyl-propylcarbamoyl)-quinoline-3-carboxylic acid.