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Granulated organoclay as a sorbent to protect the allelochemical scopoletin from rapid biodegradation in soil

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ABSTRACT

Allelochemicals have been proposed as environmentally friendly bioherbicides, but their short persistence in soils often limits their performance as natural weed management products. In this study, incorporation into organoclay granules was investigated as a strategy to protect the allelochemical scopoletin from rapid biodegradation and prolong its persistence in soil. The commercial organoclay Cloisite® 10A, in its raw powder form, was used to prepare the granules. A kinetic study revealed slower sorption of scopoletin on the granules than on the organoclay powder and indicated an intraparticle pore diffusion mechanism. The half-life of scopoletin in soil under laboratory conditions increased significantly by incorporating the allelochemical into the organoclay granules, from 0.34 to 14.4 days. A field experiment was also conducted to assess whether the increase in soil half-life measured under controlled laboratory conditions translated to field conditions and to compare the phytotoxicity of the granulated allelochemical with that of its free (dissolved) form. The addition of scopoletin-loaded organoclay granules to soil plots rendered a field half-life for the allelochemical of 20.1 d, in contrast to the value of 0.54 d obtained for its free form. The granules also favored the expression of the phytotoxicity of scopoletin, reducing germination and root growth of *Lactuca sativa* L. to a greater extent than free scopoletin. The results of this work indicate that incorporation into organoclay granules could be a suitable technological approach to provide allelochemicals with protection from rapid biodegradation losses in soil, which may help increase their persistence for a better performance as crop protection products.
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1. Introduction

In agricultural systems, weeds are a primary cause of crop yield losses since they compete for water, nutrients, light, and other growth resources (de Mastro et al., 2021; Hasan et al., 2021; Nichols et al., 2015). The intensive application of synthetic herbicides to reach a proper level of weed control has led to human health and environmental contamination problems, continuing weed resistance, and loss of soil biodiversity (Abbas et al., 2021; Cordeau et al., 2016; Gámiz and Celis, 2021; Yusà et al., 2022). Hence, there is a prevailing need to look for alternative weed management products, which should fulfill the conditions of being effective, non-contaminant, and economically affordable (Duke et al., 2019; Korres et al., 2019; Serino et al., 2021).

Allelopathy consists of the release of biochemicals by microorganisms or plants with a concomitant direct or indirect effect on the growth of neighboring plants or microorganisms in a given natural or agricultural system (Korres et al., 2019).

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Accordingly, allelopathic compounds have been suggested as a tool in the frameworks of integrated weed management and organic farming based on the fact that many of them display phytotoxic properties (Céspedes et al., 2014; de Mastro et al., 2021; Galán-Pérez et al., 2021; Gámiz and Celis, 2021; Jilani et al., 2008; Macías et al., 2019; Domingues and Santos, 2019; Trezzi et al., 2016). Nevertheless, the identification of allelopathic activity in the laboratory does not always translate to the field (Nichols et al., 2015). This is because the herbicidal activity is strongly dictated by the dynamics of the allelochemical in the soil environment, where it usually suffers transport and transformation processes that can restrain its bioactivity within hours or a few days (Bravetti et al., 2020; Gámiz et al., 2018; Gimsing et al., 2009; Macías et al., 2019).

Scopoletin belongs to the family of hydroxycoumarinic allelochemicals, for which the presence of a hydroxyl group in the C7 position has been suggested to contribute to their phytotoxicity (Pan et al., 2015). Hydroxycoumarins have been proposed as potential surrogates for synthetic herbicides because they appear to have similar activity to auxinic herbicides, such as 2,4-D or MCPA (Graña et al., 2017). In former studies, we found that the phytotoxicity of scopoletin depended on soil type and specifically on how sorption and persistence affected its bioavailability. For example, scopoletin did not express its phytotoxicity in alkaline soils because these favored a fast decrease of its soil concentration (Galán-Pérez et al., 2021, 2022). Hence, controlling the bioavailability of scopoletin in the soil environment appears a critical step for the use of this and similar hydroxycoumarins as bioherbicides.

Nanotechnology has been proposed as a tool to overcome the limitations associated with the use of natural compounds as bioherbicides (Jilani et al., 2008; Korres et al., 2019). The advantages ascribed to its use include the modulation of the release of the compound into the environment, the optimization of the application doses, and the reduction of chemical losses by volatilization or leaching, for example, through nanoformulation of the active ingredient (Vurro et al., 2019). Numerous matrixes have been proposed for this aim, such as nanoparticles, nanocapsules, nanoclays, or liposomes, which can protect the compound against degradation, volatilization, or leaching (de Mastro et al., 2021; Pérez-de Luque and Hermosín, 2013). Specifically, organically-modified clays show exceptional properties as sorbents of organic compounds (Saleh et al., 2020; Sarkar et al., 2012; Slaný et al., 2019). The commercial organophilic clay Cloisite® 10A, for instance, was found to increase the persistence of scopoletin in alkaline soil by enhancing its sorption (Galán-Pérez et al., 2022). As reversible sorption has traditionally dominated the interactions between organoclays and organic compounds (Gámiz et al., 2019; Sarkar et al., 2012), the bioaccessibility of allelochemicals sorbed on organoclays may still allow the expression of their biological activity.

One of the main disadvantages of the direct use of organoclays as sorbents or carriers lies in the fact that the handling of powdered materials is problematic because of the risk of drift and inhalation of small particles (Du et al., 2017). It has also been observed that, once in the soil, powdered organoclays may rapidly lose some of their sorption capacity (Gámiz et al., 2017). To circumvent these limitations, the granulation of organoclays to agglomerate fine particles into larger ones and improve their physical features, such as permeability, sorption, and bulk density, has been recommended (Bueno et al., 2021; Du et al., 2017; Huang et al., 2013; Jiang et al., 2015; Visavale et al., 2007). Recently, we successfully prepared granules based on Cloisite 10A and used them as carriers of the monoterpene S-carvone. The prepared granules behaved as a slow-release system, reducing transport losses of the allelochemical and prolonging its phytotoxic effect in laboratory tests (Gámiz and Celis, 2021).

Based on the hypothesis that granules of the commercial organoclay Cloisite 10A could perform better than its powder form in enhancing the persistence of the hydroxycoumarin scopoletin in alkaline soils, the objectives of this work were: (1) to compare the ability of powder and granules of Cloisite 10A to sorb the allelochemical scopoletin; (2) to evaluate the effect of different coapplication forms of the organoclay and scopoletin on the allelochemical dissipation rate in an alkaline soil under controlled laboratory conditions, (3) to evaluate the behavior of scopoletin-loaded organoclay granules under field conditions in terms of persistence and phytotoxic activity of the allelochemical. This work fundamentally advances in the interaction between organoclays and natural organic compounds and its application in the search for strategies to implement the use of allelochemicals as potential bioherbicides.

2. Materials and methods

2.1. Materials

Scopoletin (6-methoxy-7-hydroxycoumarin) was purchased from Merck (Spain) with a purity > 99.0%. The compound has a molecular mass of 192.17, pK_a of 7.4 (Ketai et al., 2001), and its experimentally-determined water solubility was 230 mg L^{-1} at 25 °C. All diluted solutions of scopoletin used in the experiments were obtained from a 200 mg L^{-1} stock solution prepared in water. Fig. 1 shows the chemical structure of scopoletin.

The soil sample used in the laboratory experiment was taken from the top 20 cm of a field area located at the CSIC Experimental Station in Seville, Spain (37°16′54.5″N 06°03′59.0″W). Once in the laboratory, it was air-dried, sieved using a 2 mm diameter sieve, and analyzed by the Soil Analysis Service of IRNAS (CSIC). The soil was a sandy loam containing 75% sand, 6% silt, 19% clay, 0.34% organic carbon, and had a pH of 8.4 (determined in a 1 g:2.5 mL soil:water slurry).

The commercial organoclay Cloisite® 10A (BYK Additives & Instruments, Wesel, Germany) was used in its raw (powdered) form for the preparation of sorbent granules. The organoclay Cloisite 10A has a basal spacing value of 1.92 nm, and its elemental analysis revealed a C content of 27.0% and a N content of 1.13%, corresponding to the presence of dimethyl, benzyl, hydrogenated alkyl tallow quaternary ammonium cations as modifiers at a loading of ca. 125 cmol kg^{-1} clay (Galán-Pérez et al., 2022). The structure of the interlayer cation and additional characteristics of Cloisite 10A can be found elsewhere (Galán-Pérez et al., 2022; Pastor et al., 2020).

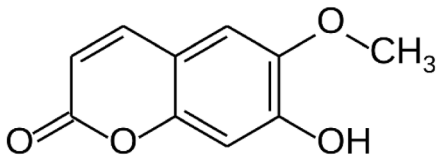

Fig. 1. Chemical structure of scopoletin.

2.2. Granulation of Cloisite 10A

The granulation procedure consisted of introducing 50 g of organoclay in a rolling drum granulator (model DGI-01, Languedoc Scientifique, Rivesaltes, France) where the organoclay was moistened with 50 mL of distilled water, used as a liquid binder for agglomeration. The water was sprayed with a nebulizer while breaking big granules with a spatula to control the snowball effect. The obtained granules were dried at 60 °C overnight and then sieved to select those with sizes between 63 μm and 2 mm.

2.3. Scopoletin sorption kinetics on powder and granules of Cloisite 10A

The kinetics of sorption of scopoletin on raw (powdered) and granulated Cloisite 10A were compared to get insight into the effect of granulation on the sorption rate and extent. For this purpose, 320 mg of Cloisite 10A, as powder or granules, were placed in 500 mL-amber glass bottles with screw caps and 250 mL of a scopoletin aqueous solution with an initial concentration of 2 mg L^{-1} were added. The bottles were incubated at 25 ± 1 °C and, at different times (t = 0, 8, 24, 48, and 72 h), aliquots of 4 mL of the supernatant solution were taken, filtered, and stabilized with 4 mL of methanol before analysis by high-performance liquid chromatography (HPLC), following the protocol for sample storage described by Galán-Pérez et al. (2021). The experiment was performed in duplicate. The amount of scopoletin sorbed at different times was calculated from the difference between the initial and final concentration of allelochemical in solution.

2.4. Scopoletin dissipation in soil under controlled laboratory conditions

The dissipation of scopoletin in soil was evaluated through an incubation experiment performed in 15 mL-Pyrex® centrifuge tubes. Four treatments were established: (1) untreated soil + scopoletin, (2) soil + raw (powdered) Cloisite 10A + scopoletin, (3) soil + granules of Cloisite 10A + scopoletin, and (4) soil + granules of Cloisite 10A preloaded with scopoletin. For treatments (1), (2), and (3), 0.3 mL of an aqueous solution of scopoletin of 30 mg L^{-1} was mixed with 1 g of soil samples, either unamended or previously amended with 20 mg of Cloisite 10A in powder or granulated form. For treatment (4), the scopoletin solution (0.3 mL, 30 mg L^{-1}) was preincubated with the granules of Cloisite 10A (20 mg) for three days, and then the suspension was mixed with the soil. Under these conditions, the initial concentration of scopoletin in all treatments was 9 mg kg^{-1} soil, the soil humidity was 30%, and the organoclay was present at 2% (20 mg per gram of soil) in treatments (2), (3), and (4). For each treatment, 21 tubes were prepared and incubated at 25 ± 1 °C in darkness after being hermetically closed. At selected times (t = 0, 0.3, 1, 2, 3, 4, and 7 days), three tubes of each treatment were taken from the incubator (SalvisLab, Rotkreuz, Switzerland) and frozen at −18 °C for subsequent extraction and analysis by HPLC. The extracting solution was the same as that reported in Galán-Pérez et al. (2021), i.e., 8 mL of 90:10 (v/v) methanol:0.01 M o-phosphoric acid (pH = 2.2), which rendered recoveries > 85%.

2.5. Preparation of granules of Cloisite 10A loaded with scopoletin for their use in the field experiment

Based on the results of the dissipation experiment, granules of Cloisite 10A preloaded with scopoletin were prepared for their use in the field experiment. The scopoletin-loaded granules were obtained by soaking 65 g of granules of Cloisite 10A in 1.5 L of an aqueous solution of scopoletin of 200 mg L^{-1}, to give an allelochemical content of 4.6 mg g^{-1} of granules. After 72 h, the supernatant solution was removed and the granules were dried at 60 °C. Extraction of 20 mg of granules with 8 mL of a 90:10 (v/v) methanol:diluted H_3PO_4 (pH = 2.2) solution revealed that their actual content in scopoletin was 4.4 ± 0.3 mg g^{-1}.

2.6. Field trial

The field experiment was performed in an area adjacent to the point where the soil sample used in the laboratory dissipation experiment was collected (37°16′60″N, 06°03′58″W). The soil was a sandy loam with 63% sand, 21% silt, 16% clay, 1.42% organic carbon, and a pH of 8.3. The trial design comprised six plots of 0.25 m^2 (0.5 × 0.5 m) demarcated with woody frames of 20 cm height with a distance among them of 0.25 m (Supplementary Fig. S1). In duplicate, three treatments randomly distributed were established: (1) two untreated soil plots (controls), (2) two soil plots treated with a scopoletin solution, and (3) two soil plots treated with organoclay granules preloaded with scopoletin as described

in Section 2.5. Treatment (1) received 1.5 L of water, treatment (2) received 1.5 L of a 200 mg L^{-1} aqueous scopoletin solution, whereas treatment (3) received 65 g of scopoletin-loaded organoclay granules plus 1.5 L of water. In this way, the application rate of scopoletin achieved for treatments (2) and (3) was 12 kg ha^{-1}. This application rate had previously been found to control scopoletin-target plant species in acid soil but not in alkaline soils (Galán-Pérez et al., 2021, 2022). The granules were mixed within the top 1 cm of soil using a rake to achieve a homogeneous distribution and yield an organoclay content of 2% (assuming a soil bulk density of 1.3 g cm^{-3}). In addition, all plots received 3 g of seeds of *Lactuca sativa* L. (Vilmorin, La Ménitré, France), which was selected as a model, scopoletin-sensitive plant species (Galán-Pérez et al., 2022) to compare the expression of the phytotoxicity of scopoletin applied to the field plots in a free (immediately available) and granulated form. For this purpose, the total number of germinated seeds and the root length of twelve emerged seedling samples were measured for each plot at the end of the experiment (t = 7 days). The application was carried out on September 27, 2021. At days 0, 2, 4, and 7 after application, triplicate subsamples for each plot were collected from the first 5 cm of soil with a 2.5 cm internal-diameter auger, until completing six observations per treatment. The soil subsamples were separately packed in plastic bags, introduced into an icebox, and, once in the lab, frozen at −18 °C until their analysis. Extraction of scopoletin was conducted by shaking (24 h) triplicate 3 g-aliquots of each soil subsample with 15 mL of the extracting solution described in Section 2.4 and the extracts were analyzed by HPLC. The water content of the soil subsamples was also measured by drying 1 g of soil at 105 °C for 24 h.

2.7. Analysis of the allelochemical

The analysis of scopoletin was conducted by HPLC using a high-performance liquid chromatograph (Waters, Barcelona, Spain) with UV detection, following the conditions detailed in Galán-Pérez et al. (2021). Briefly, the column used was a Kinetex C18 (150 mm length × 4.6 mm i.d. and 5 μm of particle size, Phenomenex, Madrid, Spain), the mobile phase was methanol:0.01 M o-phosphoric acid (45:55) at a flow rate of 1 mL min^{-1}, and quantification was carried out at a wavelength of 343 nm.

2.8. Data treatment

The Weber & Morris (W&M) intraparticle diffusion model was used to fit the scopoletin sorption kinetic data on the granules:

$$q_t = k_{WM} \cdot t^{0.5} + C \quad (1)$$

where q_t (mg kg^{-1}) is the amount of scopoletin sorbed at time t (h), k_{WM} is the W&M parameter, and C represents the boundary layer effect or surface sorption (Weber and Morris, 1963; Schwaab et al., 2017).

Laboratory and field soil dissipation data were described by a first-order equation:

$$C_s = C_0 . e^{-k \cdot t} \quad (2)$$

where C_s (mg kg^{-1}) is the concentration of scopoletin in soil at time t (days), C_0 (mg kg^{-1}) is the concentration of scopoletin in soil at t = 0, and k is the first-order dissipation rate constant, which was used to calculate the soil half-lives of scopoletin as $t_{1/2} = (\ln 2)/k$.

In the sorption, dissipation, and seed germination experiments, standard errors were used to indicate variability among replicates. Significant differences were established at the 95% confidence level using the t-test, when two treatments were compared (sorption and field dissipation data), or analysis of variance (ANOVA) followed by LSD post-hoc test, when more than two treatments were compared (dissipation in the laboratory and seed germination in the field). Data distribution for the root length of emerged seedling samples in the field experiment was analyzed using box and whisker plots.

3. Results and discussion

3.1. Scopoletin sorption kinetics on powder and granules of Cloisite 10A

The amount of scopoletin sorbed on powder and granules of Cloisite 10A as a function of time during the sorption kinetic experiment is shown in Fig. 2a. The rapid sorption of the allelochemical on the powdered organoclay contrasted with its slower sorption on the granules. For the powder, there was almost immediate sorption of ca. 50% of the allelochemical initially present in solution, and the maximum uptake (86%, 1340 mg kg^{-1}) was reached within 8 h, yielding a sorption distribution coefficient K_d = 4800 L kg^{-1}. The amount of scopoletin immediately sorbed by the granules was negligible, and only after 72 h, sorption became similar to that observed by the powder (Fig. 2a). Interestingly, the scopoletin sorption kinetic data on the granules were very well described by an intraparticle diffusion model (Eq. (1)), with the plot of the amount of scopoletin sorbed (q_t) against $t^{0.5}$ giving a straight line (k_{WM} = 125±4 mg kg^{-1} $h^{-0.5}$, R^2 = 0.997) that passed through the origin (Fig. 2b). This strongly indicated that intraparticle diffusion was the rate-controlling process in the sorption of scopoletin on the granulated organoclay (Wang and Guo, 2020). Formerly, we had observed that raw (powdered) Cloisite 10A was an excellent sorbent for scopoletin from aqueous solution (Galán-Pérez et al., 2022). Hydrophobic interactions between scopoletin and the alkyl chains of the organoclay supplemented by polar interactions between scopoletin anions and the positively charged ammonium groups of the organic modifier were proposed as potential interaction mechanisms (Galán-Pérez et al., 2022). It appears that the major effect of granulating the organoclay was to reduce the availability of sorption sites, with intragranule diffusion becoming the sorption rate-controlling step.

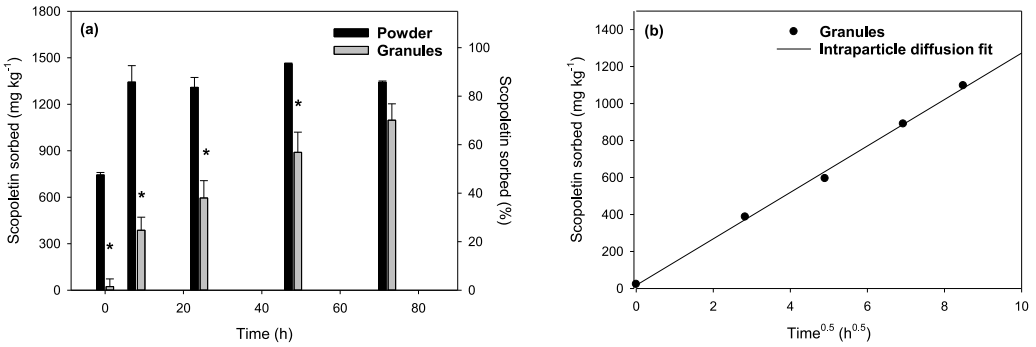

Fig. 2. Scopoletin sorbed on powder and granules of Cloisite 10A as a function of time during the sorption kinetic experiment (a) and fit of the sorption kinetic data obtained for the granules by the Weber & Morris intraparticle diffusion model (b). The asterisk indicates that the difference in sorption is statistically significant at the $P < 0.05$ level.

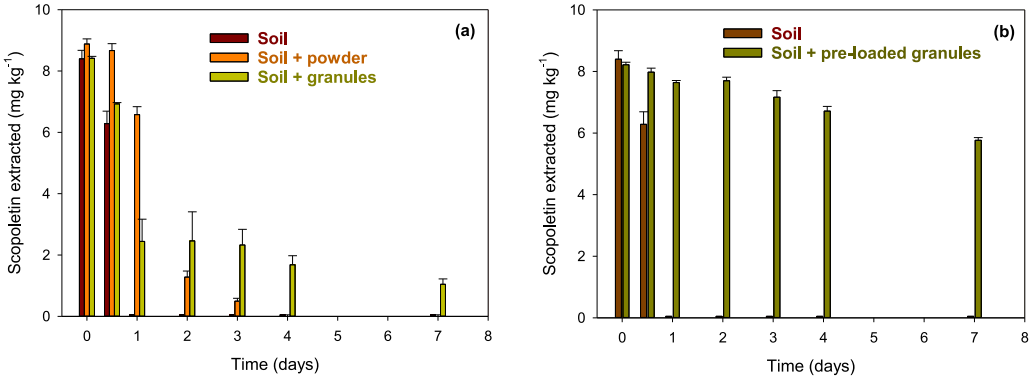

Fig. 3. Scopoletin dissipation data in soil treated with powder and granules of the organoclay Cloisite 10A (a) and in soil treated with granules of the organoclay preloaded with the allelochemical (b) under laboratory conditions.

3.2. Dissipation of scopoletin in soil under laboratory conditions

The effect of powder and granules of Cloisite 10A on the dissipation of scopoletin in soil under controlled laboratory conditions is summarized in Fig. 3 and detailed in Supplementary Table S1. Scopoletin was added either as an immediately available aqueous solution of allelochemical (for the "soil", "soil + powder", and "soil + granules" treatments) or as allelochemical-preloaded organoclay granules (for the "soil + preloaded granules" treatment). The parameters obtained after fitting a first-order dissipation equation to the experimental data are given in Table 1.

Scopoletin dissipation data reflected a very rapid disappearance of the allelochemical in untreated soil ($k = 2.059$ d^{-1}, $t_{1/2} = 0.34$ d), with scopoletin residues decreasing to non-detectable levels just one day after its application to the soil (Fig. 3 and Supplementary Table S1). Allelochemicals commonly display a fast dissipation in soils because their (natural) chemical structures are readily degradable by soil microorganisms (Dalton et al., 1989; Inderjit, 2005). Indeed, we have previously shown that the degradation of scopoletin in soil is predominantly a rapid, microbial-mediated process, particularly in soils with low sorption capacities, where the high fraction of allelochemical remaining in the soil solution can be readily degraded by soil microorganisms (Galán-Pérez et al., 2021, 2022).

All organoclay treatments increased the persistence of scopoletin in the soil ($P < 0.05$) (Supplementary Table S1), but the specific dissipation patterns were strongly dependent on the form the organoclay and allelochemical were added to the soil (Fig. 3, Table 1). A key observation was that the organoclay powder was more effective than the granules in increasing the soil persistence of scopoletin at short incubation times ($t \leq 1$ day) while this effect was reversed at longer incubation times ($t \geq 2$ days) (Fig. 3a and Supplementary Table S1). Even though this behavior resulted in similar half-lives for scopoletin in the "soil + powder" and "soil + granules" treatments ($t_{1/2} = 0.99-1.17$ d, Table 1), it indicated that the high availability of (external) sorption sites in the powdered organoclay (Fig. 2) led to a rapid protection from biodegradation, but also to a high bioaccessibility of the sorbed fraction to be subsequently degraded by soil microorganisms (Semple et al., 2004). The reduced availability of sorption sites in the granules led to slower sorption and a concomitant lower protection at short incubation times but, once a fraction of the allelochemical reached the (intraparticle) sorption sites, it became strongly protected from degradation (Fig. 3a). Highly reversible, external surface sorption of allelochemicals in soil

Table 1
First-order laboratory dissipation constants for scopoletin in untreated soil and in soil treated with powder, granules, and preloaded granules of the organoclay.

	k ($days^{-1}$)	$t_{1/2}$ (days)	R^2
Soil	2.059 ± 0.449[a]	0.34 (0.28–0.43)[a]	0.969
Soil + powder	0.699 ± 0.153	0.99 (0.81–1.27)	0.941
Soil + granules	0.591 ± 0.161	1.17 (0.92–1.61)	0.863
Soil + preloaded granules	0.048 ± 0.004	14.4 (13.3–15.7)	0.971

[a]Standard error range of the calculated coefficients.

Table 2
First-order dissipation constants for scopoletin applied to field plots as a free compound or presorbed on organoclay granules.

	k ($days^{-1}$)	$t_{1/2}$ (days)	R^2
Free scopoletin	1.282 ± 0.305[a]	0.54 (0.44–0.71)[a]	0.992
Granules	0.034 ± 0.003	20.1 (18.4–22.3)	0.983

[a]Standard error range of the calculated coefficients.

amended with powdered organoclays has previously been described and shown to promote rapid loss of the organoclay sorption capacity with time (Gámiz et al., 2017; Real et al., 2019).

The soil dissipation pattern for the scopoletin-preloaded organoclay granules confirmed the afore-mentioned protection mechanism, as it revealed that scopoletin already sorbed on intraparticle sorption sites of the granulated organoclay dissipated very slowly in the soil (Fig. 3b). The half-life of scopoletin increased from 0.34 d, when applied as an immediately available aqueous solution to the soil, to 14.4 d, when applied as allelochemical-preloaded organoclay granules (Table 1). For the preloaded granules, at $t = 7$ d, scopoletin residues still represented 70% of the amount of allelochemical initially added to the soil. Intraparticle pore diffusion of scopoletin hampered desorption, delaying biodegradation and increasing the persistence of scopoletin in the soil.

3.3. Field experiment

Weather conditions monitored during the field experiment are compiled in Table S2 of the Supporting Information. The maximum daily temperatures varied between 23.1 and 32.3 °C, with an average of 29.5 °C, whereas the minimum daily temperatures ranged between 12.0 and 15.2 °C, with an average of 14.1 °C (Supplementary Table S2). Warm temperatures and the absence of rain made the soil plots become noticeably dry during the experiment whereby, daily, we moistened them with 3 mm of water to facilitate the soil sampling and favor seed germination. At $t = 7$ d, this was not necessary since a natural rainfall episode of a similar amount of water occurred shortly before sampling (Supplementary Table S2).

The soil concentrations of scopoletin (mg kg^{-1} dry soil) applied to the plots as free (dissolved) compound or pre-sorbed on granules, measured at different sampling times after application, are shown in Fig. 4. A first observation was that the initial concentration (t = 0) of scopoletin in soil differed notably between treatments (Fig. 4). For the plots treated with the free compound, the amount of allelochemical extracted at $t = 0$ was 5.2 ± 0.3 mg kg^{-1}, whereas a value of 26.7 ± 4.0 mg kg^{-1} was obtained for the plots treated with the granules. Considering the bulk density (1.3 g cm^{-3}) and humidity (12%) of the soil samples collected at t = 0, we expected a concentration of ca. 21 mg kg^{-1}, a value close to that obtained for the plots treated with the granules but much greater than that measured for the plots treated with the free allelochemical (Fig. 4). The most likely explanation for this result is that scopoletin, added as a free compound, biodegraded very rapidly as soon as it reached the soil, a behavior previously observed for other allelochemicals (Real et al., 2021). Interestingly, the application of scopoletin preloaded on organoclay granules was very efficient in preventing this rapid initial loss of allelochemical.

Fig. 4 shows that the dissipation of scopoletin in the field occurred at a slower rate when it was applied pre-sorbed on granules compared to the free compound (Fig. 4 and Table 2). For instance, seven days after treatment, we measured a soil concentration of scopoletin of 21.1 ± 4.2 mg kg^{-1}, compared to the value of 0.16 ± 0.08 mg kg^{-1} obtained for the free compound (Fig. 4). The half-life of free scopoletin in field plots ($t_{1/2} = 0.54$ d) was similar to that measured in the laboratory experiment ($t_{1/2} = 0.34$ d) (Tables 1 and 2). Likewise, the persistence of scopoletin applied as organoclay granules under field conditions ($t_{1/2} = 20.1$ d) was only slightly higher than that measured under laboratory conditions ($t_{1/2} = 14.4$ d). Thus, the dissipation patterns observed under controlled laboratory conditions were reproduced in the field. It should be taken into account that a slower release of the allelochemical could have occurred in the field because of less favorable (fluctuating) temperature and humidity conditions for the release of the bioherbicide than under controlled laboratory conditions, and that temperature and water content changes could have also impacted the activity of soil microbial degraders (Chowdhury et al., 2021; Galán-Pérez et al., 2021; Real et al., 2021). Based on the weather conditions occurring during the field experiment (Supplementary Table S2), it is not likely that processes such as leaching or runoff, which often play a relevant role in the field dissipation of agrochemicals, contributed significantly to the dissipation of scopoletin in our field experiment.

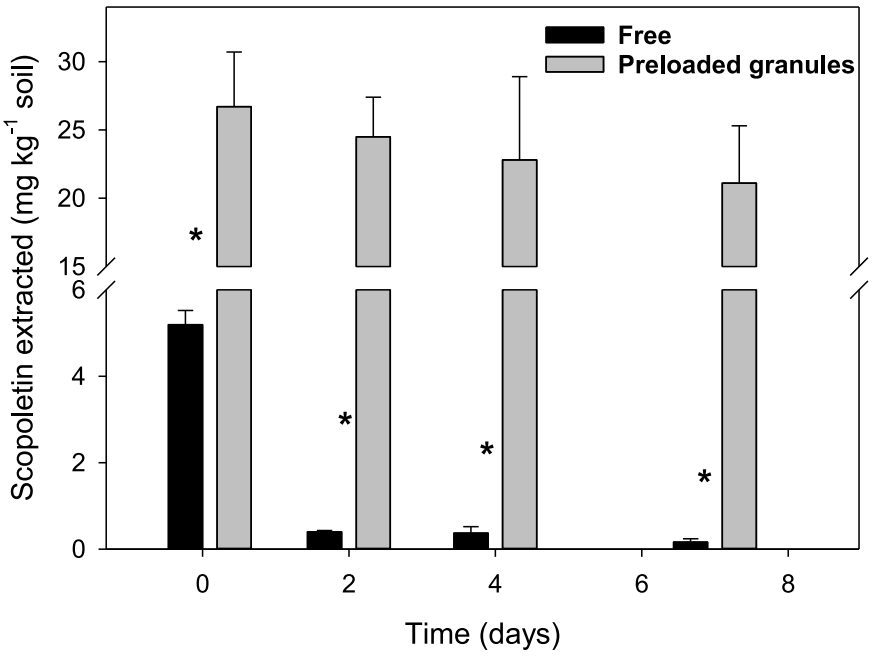

Fig. 4. Dissipation data for scopoletin applied to field plots as a free compound or presorbed on organoclay granules. The asterisk indicates that the difference in the soil concentration of scopoletin is statistically significant at the $P < 0.05$ level.

The number of germinated seeds and the root length of emerged seedling samples of *Lactuca sativa* in the plots treated with free scopoletin and with scopoletin pre-sorbed on organoclay granules were compared with the values obtained for untreated control plots (Fig. 5). The aim was to address the question of whether the extended persistence of the allelochemical in the granulated formulation favored the expression of its phytotoxicity in the soil. In contrast to previous laboratory bioassays reporting negligible effects of scopoletin on germination of target plant species (Galán-Pérez et al., 2021; Graña et al., 2017), we observed that scopoletin affected the germination of *Lactuca sativa* seeds in field plots, particularly when it was applied as organoclay granules, where a reduction in seedling emergence of 80% was observed (Fig. 5a). A possible explanation for this finding is that the effect of scopoletin on germination became more apparent under less favorable, field conditions than under laboratory conditions, as the latter are often optimized to favor seedling emergence.

The data distribution for the root length of emerged seedling samples was analyzed using box and whisker plots (Fig. 5b). Medians and means for root length values decreased in the following order: Control $\geq$ Free scopoletin $>$ Preloaded granules (Fig. 5b). Interestingly, the median and mean for the plots treated with the free allelochemical (5.1 and 4.9 cm, respectively) were similar to those for the control plots (5.0 and 5.3 cm), whereas the plots treated with the granules preloaded with scopoletin yielded statistically significant lower values of 3.8 cm and 3.5 cm (Fig. 5b).

For the control plots, a positively skewed box with upper/lower quartiles at 7.0 and 3.8 cm was observed, whereas for the granules, a negatively skewed box with upper/lower quartiles at 4.7 and 2.0 cm was obtained. As we previously checked that the granules themselves had no significant effect on germination or growth of *Lactuca sativa* after their application to the tested soil (Supplementary Fig. S2), the reduction in germination and root growth observed for scopoletin applied as granules, compared to the free form of the allelochemical, was attributed to the greater persistence of the allelochemical when it was applied in granulated form (Fig. 4).

4. Conclusions

The interaction of scopoletin with granules of the organoclay Cloisite 10A satisfactorily protected the allelochemical against rapid biodegradation in an alkaline soil, both under laboratory and field conditions. A superior protective effect was observed when scopoletin was pre-sorbed on the organoclay granules before being applied to the soil. Intraparticle diffusion through the granules limited the availability of the allelochemical to soil microorganisms, increasing its soil persistence and favoring the expression of its phytotoxicity under a real environmental conditions in the field.

The outcomes from this study support that incorporation into granulated nanoclays can be a useful technological approach to increase the persistence and bioactivity of allelochemicals in soils. Nevertheless, to translate our findings to a real weed management agricultural scenario, the proposed formulation would need to be optimized in terms of cost and bioactivity to specific target weeds, with a particular focus on minimizing the amount of allelochemical

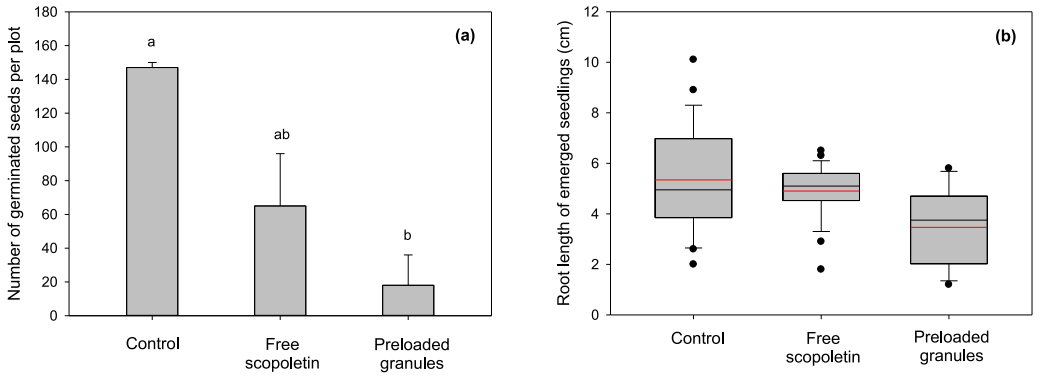

Fig. 5. Effect of scopoletin applied to field plots as a free compound or presorbed on organoclay granules on germination (a) and the root length of emerged seedlings (b) of *Lactuca Sativa* L. Different letters above the seed germination bars indicate statistically significant differences at the $P < 0.05$ level. For root lengths, boxes indicate the limits for the upper and lower quartiles, whiskers indicate the 90th and 10th percentiles, symbols represent outlying data, and black and red lines inside the boxes correspond to median and mean values, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and granulated carrier per hectare needed for weed control. The results of previous works demonstrating a strong phytotoxic effect of scopoletin to weed species such as *Orobanche crenata* and *Arabidopsis thaliana* under soilless laboratory conditions (Fernández-Aparicio et al., 2013; Graña et al., 2017) encourage higher-tier assessments directed to optimize the formulation proposed in this work for practical use. As a possible matter of further investigation, increasing the content of scopoletin in the granules would not only reduce the amount of granulated formulation required to achieve the same herbicide application rate, but could also increase the herbicidal activity of the allelochemical in the formulation, as herbicide molecules present at high loadings in organoclays are commonly more loosely bound than those present at low loadings (Celis et al., 2005). Further potentially useful optimization approaches include tuning the nature of the organoclay and the physical characteristics of the granules, such as their particle and pore size. This might contribute to the standardization of strategies for the implementation of allelochemicals as potential bioherbicides.

CRediT authorship contribution statement

Jose Antonio Galán-Pérez: Formal analysis, Investigation, Visualization, Writing – original draft. **Beatriz Gámiz:** Conceptualization, Formal analysis, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Rafael Celis:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2022.102707.

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