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S-Carvone Formulation Based on Granules of Organoclay to Modulate Its Losses and Phytotoxicity in Soil

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Abstract: Based on the effects that allelochemicals can exert over organisms, their use as alternatives to synthetic pesticides has been proposed. To this aim, it is important to understand their behavior in soils as allelochemicals can readily dissipate by different routes. In this work, novel granules based on the commercial organoclay Cloisite® 10A were prepared as a new strategy for the possible application of S-carvone as a bioherbicide, overcoming its rapid dissipation in the environment. Batch release, degradation, mobility, and phytotoxicity tests in soil were performed. Until now, the phytotoxicity of organoclay-based formulations of S-carvone in soil has not been studied. The release of S-carvone in water from the granules occurred slowly. There were no differences in the persistence of the allelochemical after its application to soil as a free compound (readily available form) or supported on granules. However, the granulated formulation reduced and delayed the leaching of S-carvone, thus controlling its downward movement in soil columns, as compared to the free S-carvone. Bioassays revealed that S-carvone supported on granules reduced the germination and aerial biomass of *Lactuca sativa* L. to a greater extent than the free compound. Our results demonstrated that the prepared formulation of S-carvone, based on granules of the commercial organoclay Cloisite® 10A, could be used to control transport losses, such as leaching or volatilization, increasing the bioefficacy of the allelochemical. These findings could inspire further investigations for the preparation of novel formulations of monoterpenes as potential bioherbicides.

Keywords: allelochemicals; biopesticides; degradation; leaching; monoterpenes; organoclays; soil

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1. Introduction

The intensification of agriculture to achieve adequate crop yields has led to the massive application of synthetic pesticides, which has resulted in contamination problems by the widespread presence of their residues in the environment. Furthermore, repeated pesticide applications lead to gradual pest resistance and adverse effects on soil biodiversity [1–3]. Hence, new alternative management strategies are required to overcome these problems and ensure sustainable agricultural production. Accordingly, modern agriculture is evolving towards natural-based solutions [4].

Many natural products released by living organisms have a positive or negative influence on neighboring living organisms, a phenomenon known as allelopathy [5]. The use of allelochemicals as active ingredients in formulations for crop protection has been proposed, considering that allelochemicals can be potential eco-friendly substitutes or complements to synthetic pesticides [6–9]. Given that herbicidal, fungicidal, and insecticidal properties have been ascribed to many allelochemicals [10], these could help decrease the amounts of conventional pesticides introduced into the environment, reducing problems related to contamination, toxicity, and pest resistance [11].

Monoterpenes (C10 unsaturated hydrocarbons derived from two C5 isoprene units) are volatile organic compounds of biogenic origin and are major allelopathic components of plant (essential) oils [12]. Phytotoxicity has been one of the most studied facets of these compounds for their potential use as biopesticides [9,13–18]. However, despite the pesticidal properties attributed to monoterpenes, their commercialization as biopesticides is still very poor. One of the main limitations associated with their use lies in the difficulty of predicting their effectiveness once they reach the soil environment [11,18,19]. They can be transported by leaching to deeper soil layers or groundwater, volatilized, or chemically and/or biologically degraded [8,20]. Thus, the effect of different field conditions, such as UV radiation, humidity, pH, and inherent soil characteristics, on their stability needs to be solved [21,22] since they can be easily deteriorated by oxygen, light, moisture, heat, and microorganisms [23], leading to a reduction in bioavailability and bioactivity [9]. For instance, Karamanoli et al. [16] studied the decomposition of essential oils in soils and established monoterpene biodegradation profiles with time. No losses by transport (leaching) or chemical degradation were observed. Some compounds, such as 1,8-cineole, with high phytotoxic activity under laboratory conditions, show little activity in the presence of soil as a result of a low bioavailability [24]. These aspects suggest that monoterpenes may lack the necessary persistence/availability to be effective bioherbicides [25].

Nanoformulation is a novel approach recently proposed to modulate the rate of release of allelochemicals into the environment, specifically for monoterpenes [25]. These formulations can regulate the delivery of the active ingredient at the specific place of action, diminish the toxic effects on non-target organisms, and avoid dissipation of the active molecule by microorganisms [17,26], besides evading chemical losses by volatilization or leaching. Ouédraogo et al. [27] found that loading the cellulose and cellulose acetate with terpinen-4-ol and carvone, respectively, could efficiently prolong the retention of these compounds, retarding their volatilization. More recently, Campos et al. [28] observed reduced toxicity to non-target organisms when carvacrol and linalool were encapsulated in β-cyclodextrin-chitosan composites, while the formulation prepared displayed insecticidal activity against corn earworm (*Helicoverpa armigera*) and spider mite (*Tetranychus urticae*). These represent some examples of the current interest in the development of natural pesticides based on sophisticated formulation technologies to optimize their effectiveness. Nevertheless, little is known regarding the ability of these types of formulations to reduce monoterpenes' transport losses in soils and more research is needed in this sense.

Naturally occurring clay minerals have been broadly proposed as sorbents of pesticides due to their surface features [29]. Specifically, organoclays, which are the nanohybrid materials resulting from the intercalation of a surfactant or other organic cations or polymers [29–31] into the interlayer of swelling clay minerals, are among the most studied pesticide sorbents [32,33]. The exchange of hydrated, inorganic cations for organic ones changes the original hydrophilic surfaces of clays to hydrophobic, yielding materials with wide environmental applications and biocompatibility to be used in cosmetics, pharmaceutics, and medicine [29,34–37]. For example, clays can be selectively modified to sorb a particular pesticide, choosing the chemical characteristics of the modifier [29,38,39]. Accordingly, organoclays show exceptional properties to be used as filters for water decontamination [40], soil amendments for contaminant immobilization [41], or supports for the slow release of agrochemicals [35]. In organoclay-based pesticide formulations, the pesticide availability is controlled, minimizing undesirable transport losses, reducing possible toxicity on non-target organisms, and preventing extensive dissipation of the agrochemical [42].

In previous works, we successfully employed organoclays to regulate the behavior of carvone enantiomers in soil [8]. The organoclays satisfactorily sorbed and prevented the rapid biodegradation and leaching of the monoterpene carvone when the material was added to soil as an amendment. Furthermore, it has been verified that the sorption of natural compounds on organoclays commonly occurs through surface and reversible sorption mechanisms [8,43]. Based on this knowledge, organoclays emerge as good candidates to test their ability to control the release of this allelochemical.

Although their fine particle size could complicate the manipulation and performance of organoclays in practical applications, several works have proposed their granulation to improve their physical features [40,44,45]. The conversion of clay powders into granules could enhance the permeability and delay desorption because of the porous structure and bulk density of the granulated material [46]. Likewise, the management of the material becomes easier by avoiding segregation of fine particles during use [47]. Only a few works have evaluated the sorption of terpenes on organoclays and, more importantly, the performance of organoclay-based formulations for the slow release of monoterpenes and their subsequent herbicidal activity in soil have not been previously studied [48,49]. Accordingly, we hypothesized that the formulation of the herbicidally-active carvone enantiomer, S-carvone, as granules of organically modified clay would perform better than the allelochemical applied as a free compound. Hereafter, the aims of this work were: (i) to prepare novel granules of a commercial organoclay, Cloisite® 10A, for their use as supports for the monoterpene S-carvone, (ii) to examine the release, degradation, and mobility of S-carvone applied to soil as a formulation based on organoclay granules, and (iii) to test the phytotoxicity in soil of S-carvone supported on granules as compared to the free compound.

2. Materials and Methods

2.1. S-Carvone, Organoclay and Soil

S-carvone with analytical standard quality (purity of 98.5%, vapor pressure of 1900 mPa and solubility in water of 27 mg/L at 20 °C) [50], was provided by Sigma-Aldrich (Madrid, Spain) and used for the preparation of the granule formulation. A stock solution of 10,000 mg/L of S-carvone was also prepared in methanol to be used in the dissipation, leaching, and bioassay experiments. The schematic structure of S-carvone is represented in Figure 1.

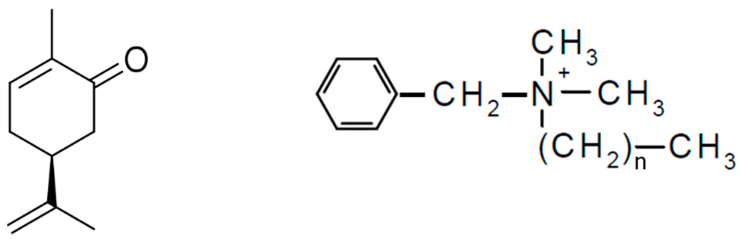

Figure 1. Schematic structure of S-carvone (**left**) and the organic modifier in Cloisite® 10A (**right**).

Cloisite® 10A, provided by BYK-Chemie GmbH (Wesel, Germany), was used as a sorbent for the monoterpene. This organoclay contains dimethyl, benzyl, hydrogenated alkyl tallow quaternary ammonium cations as organic modifier. The structure of the interlayer cation is depicted in Figure 1. The characterization of Cloisite® 10A can be found elsewhere [51].

The soil was sampled from the research station of CSIC sited in Seville (Spain) (37°16′54.5″ N 6°03′59.0″ W). A sample of the soil was obtained from the 0–20 cm surface layer, then sieved through a mesh of 2 mm and stored at 4 °C. The soil was characterized by the Soil Analysis Service of IRNAS (CSIC) as sandy clay loam soil with 74% sand, 4% silt, 22% clay, 0.6% $CaCO_3$, 0.17% organic carbon, and pH of 7.9, measured in 1:2 (*w*/*v*) soil/distilled water mixture.

2.2. Granulation Procedure

Cloisite® 10A was granulated in a Languedoc Scientifique (LSCI) granulator, model DGI-01, mixing 50 g of organoclay with 100 mL of ethanol, which was added with a nebulizer. To get the desired granule size, avoiding the snowball effect, the mixture was stirred with a spatula during spherification. Once formed, the granules were dried in an oven at 80 °C overnight and sieved to separate the particles with sizes between 63 µm and 2 mm. In Figure S1, photographs of the original powder and prepared granules of Cloisite® 10A are compared.

2.3. Preparation of the S-Carvone Formulation

The formulation of S-carvone based on organoclay was prepared by impregnating the allelochemical on the granules. To this aim, 62 mg of pure S-carvone in liquid form was added to 2 g of organoclay granules. To obtain a uniform distribution of the allelochemical on the granules, these were also impregnated with 200 µL of methanol. The formulation was allowed to air-dry for 24 h. In triplicate, 15 mg aliquots of the sample were extracted with 10 mL of methanol and analyzed by high-performance liquid chromatography (HPLC) to confirm the content in S-carvone of the formulation. Recoveries were >90%, as determined in [21]. The amount of S-carvone in the formulated granules was 3.0 ± 0.1%.

2.4. Batch Release Kinetics

A batch release kinetic study was performed to evaluate the goodness of the prepared formulation for the slow release of the allelochemical. The experiment consisted of adding 0.5 mg of S-carvone (active ingredient, a.i.) supported on granules (17.3 mg) to 250 mL of deionized water in amber glass jars closed with screw caps. At certain time intervals, the jars were agitated gently and allowed to settle down for 20 min. After that, 5 mL of solution was taken, filtered, and the concentration of S-carvone was determined by HPLC. The experiment was performed in duplicate. The amount of S-carvone released from the granulated formulation was calculated with respect to the total amount added to obtain the cumulative release values with time.

2.5. Dissipation of S-Carvone in Soil

The soil dissipation of S-carvone supported on granules was compared with that of the free, non-granulated compound through an incubation experiment. Samples of 5 g of soil were weighed in Pyrex® glass tubes, and an appropriate amount of S-carvone was added to reach a dose of 100 mg/kg soil, either as free compound (50 µL of 10,000 mg/L methanolic solution) or granulated formulation (17 mg). Next, the soil water content was adjusted near to the water holding capacity of the soil (30%) by the addition of 1.5 mL of water to the tubes. Then, the tubes were closed and incubated in darkness at 25 ± 2 °C for 14 days. Triplicate tubes were removed at selected times and frozen until analysis. S-carvone residues were extracted from the soil samples with 8.5 mL of methanol after overnight shaking. The extracts were centrifuged, filtered, and the S-carvone residues were determined by HPLC. Preliminary tests indicated that recoveries were >90%.

2.6. Leaching of S-Carvone in Soil Columns

Leaching was studied in soil glass columns (dimensions: 30 cm long × 3 cm inner diameter). The base of the columns was packed with 10 g of sea sand and glass wool to avoid soil losses during the experiment, and then 160 g of air-dry soil was used to fill the column (20 cm). Lastly, 10 g of sea sand was added on the topsoil to facilitate the uniform circulation of the allelochemical and flux of water during the experiment. Before treatment, the columns were saturated by the addition of 100 mL of distilled water, and then allowed to drain for 24 h. The water leached was measured to calculate the amount of pore volume (Vp = 60 ± 1 mL). Next, 3.6 mg of S-carvone was added to the top of the columns, either as free compound (360 µL of a 10,000 mg/L methanolic solution) or as granulated formulation (125 mg). This application rate corresponded to ca. 100 mg/kg soil considering a soil depth of 5 cm. Fifteen mL of water was added, twice a day, to the columns, simulating a rainfall episode of 40 mm/day, and the leachates were collected in vials with 5 mL of methanol to stabilize the compound and stored at 4 °C in the dark until analysis. At the end of the experiment, the columns were sectioned in segments of 5 cm (0–5, 5–10, 10–15, and 15–20 cm) which were extracted using methanol (100 mL) to obtain the amount of S-carvone remaining at different depths.

2.7. Bioassays

The phytotoxicity of S-carvone supported on granules was compared to that of the free compound by means of a soil bioassay. The bioassay was conducted in plastic pots of 20 cm^2 containing 20 g of soil, with glass wool and 30 g of sea sand positioned at the bottom to prevent soil losses. The pots were treated with 6 mL of deionized water (30% soil humidity) and, in triplicate, with 200 μL of the 10,000 mg/L methanolic solution of S-carvone or 70 mg of granules containing S-carvone at 3%. This corresponded to an application rate of 10 kg/ha and 100 mg/kg soil. Blanks with 200 μL of methanol or granules without the allelochemical were also prepared as controls. Then, 12 seeds of lettuce (*Lactuca sativa* L.) were sown on the pot surface. The samples were introduced in a growth chamber at 25 ± 2 °C, 70 ± 10% humidity, and photoperiod of 16:8-h light–dark cycles. After 7 days, plant leaves and shoots were cut and weighed, and the root length was measured.

2.8. Analytical Method

The concentration of S-carvone in solution was determined by HPLC using UV detection. The column used was a Chiralpak IG (150 mm length × 4.6 mm internal diameter and 3 μm particle size) and the analytical conditions were: acetonitrile/water (50/50) as mobile phase, flow rate of 1 mL/min, 50 μL as injection volume, and UV detection at 236 nm. Different solutions between 0.1 and 6 mg/L concentrations were employed to generate the external calibration curves.

2.9. Statistical Analysis

Standard errors were employed to express variability among replicates. Bioassay data were subjected to one-way ANOVA and subsequent post hoc tests (Tukey's HSD) for pairwise comparison between the free compound or S-carvone formulation treatments. Significant differences were established at 95% confidence level.

3. Results and Discussion

3.1. S-Carvone Release from Granules

The release of S-carvone from the prepared granules elapsed gradually during the experiment, suggesting the feasibility of the granules to act as supports for the slow release of the allelochemical (Figure 2). A plateau was identified at 4 days of the experiment, with 80% of the active ingredient released. The portion of S-carvone that remained in the granules was, most likely, trapped in the pores or strongly sorbed on the surface of the organoclay. In this regard, we formerly found weak and reversible interactions between racemic carvone and the organoclay cetyltrimethylammonium (CTMA)-Arizona montmorillonite [8], whereby difficulties in the desorption of carvone due to pore diffusion could have occurred. Additionally, the distribution coefficients calculated at this plateau ranged between 2.7 and 3.5 L/g, which were in good agreement with those reported for other organoclays used as supports for the slow release of different herbicides, such as diuron or imazamox [42,52].

The release data of S-carvone in water were fit to the widespread model [53], $Mt/Mz = kt^n + c$, to calculate the time at which the active ingredient was released at 50% (t_{50}), where Mt/Mz is the released amount (%) of the compound at time t, and k, n, and c are constants that are distinctive of the system. The calculated t_{50} was 0.8 days, which suggests the proper performance of the prepared granules as S-carvone was not released immediately into water even at a very low organoclay-to-water ratio. Additionally, the diffusional exponent, n, was 0.24 ± 0.08, which, being lower than 0.5, was indicative that the release of S-carvone into water corresponded with a Fickian diffusion mechanism [44].

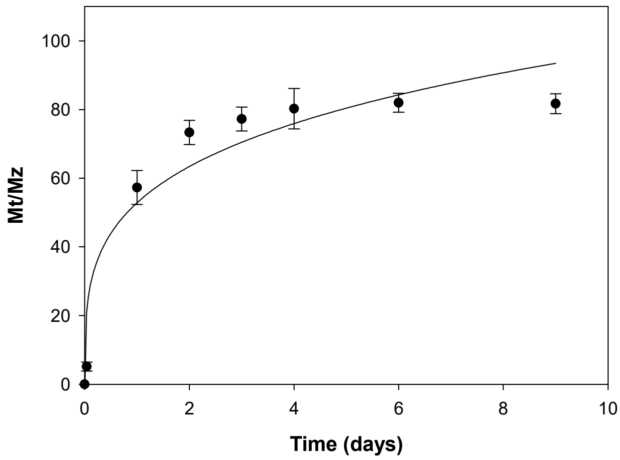

Figure 2. Release of S-carvone into water from the prepared granules. Symbols are experimental data points whereas the line is the fit to the equation $Mt/Mz = kt^n + c$.

3.2. Dissipation of S-Carvone in Soil

The dissipation data of S-carvone applied to soil either as free compound or supported on the granules are shown in Figure 3. Data were fairly defined by the first-order dissipation kinetics, with $R^2 > 0.906$ (Table 1). According to Figure 3 and data in Table 1, S-carvone presented similar half-lives ($p > 0.05$) when it was applied as free compound or supported on granules (30–40 days). This suggested that the formulation in granules did not have an adverse impact by prolonging excessively the persistence of the monoterpene in the soil. A similar behavior had previously been described for the herbicide diuron supported on powder of the organoclay SA-HDTMA [42].

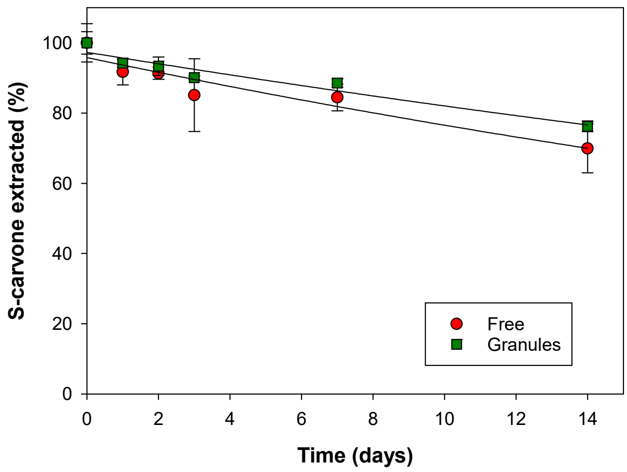

Figure 3. Dissipation curves for S-carvone applied to soil as free compound or supported on granules of Cloisite® 10A. Symbols denote experimental data points and lines are the dissipation curves fitted by the first-order kinetics. Error bars designate the standard errors of triplicates.

Table 1. Dissipation constants (k), half-lives ($t_{1/2}$), and R^2 for the dissipation of S-carvone in soil after its application as free compound or supported on granules of Cloisite® 10A.

Treatment	k (Days^{-1})	$t_{1/2}$ (Days)	R^2
S-carvone (Free)	0.0224 ± 0.0038	30 (26–37)	0.906
S-carvone formulation	0.0171 ± 0.0023	40 (36–47)	0.936

Interestingly, the half-lives obtained in this work for S-carvone contrasted with those previously found by Gámiz et al. [8,21] for racemic carvone in similar Mediterranean soils, where much shorter half-life values between 0.7 and 0.8 days were obtained. It is important to note that, in this work, we used a different methodology and application rate in the dissipation experiment compared to those used in Gámiz et al. [8,21]. In particular, the dose of 100 mg/kg of S-carvone used here was greater than that of 2 mg/kg of racemic carvone used in Gámiz et al. [8,21]. To clarify whether the application dose could impact the dissipation rate of S-carvone, we repeated our incubation experiment at a dose of 2 mg/kg. The results showed a change in the degradation pattern (Figure S2), which became similar to that reported in Gámiz et al. [21], yielding a calculated half-life value of 1.4 days and evidencing that the persistence of carvone is prominently concentration-dependent. The leading degradation mechanism of the monoterpene in soil has been proposed to be microbial-mediated [21]. Additional processes, such as volatilization or photodegradation, can account for its dissipation in soils, but in our incubation experiment, losses by these processes can be discarded since the experiments were carried out in sealed tubes and in the dark. Therefore, some toxic effects on soil microorganisms could have enhanced the persistence of S-carvone in the soil at higher doses [54], which becomes an interesting subject of further investigation.

3.3. Leaching of S-Carvone in Soil

The breakthrough curves (BTCs), relative and cumulative, of S-carvone after its application as a free compound or supported on the granules to the soil columns are shown in Figure 4.

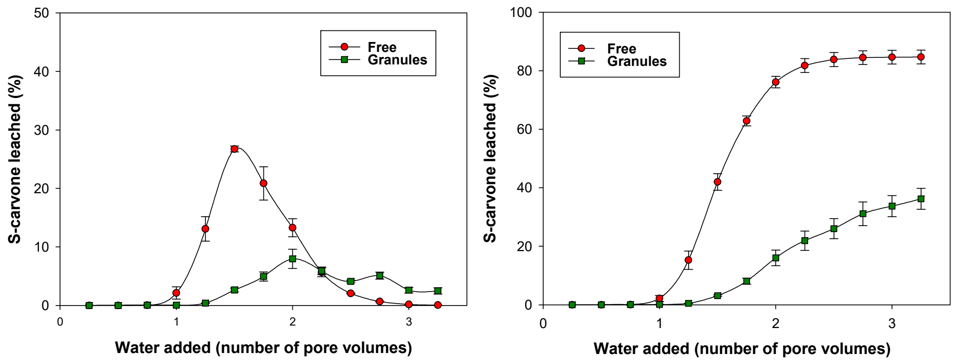

Figure 4. Breakthrough curves (BTCs) for S-carvone applied to soil columns as free compound or supported on granules: Relative curves (**left**) and cumulative curves (**right**).

Different leaching patterns of S-carvone depending on the application form are inferred from the registered BTCs (Figure 4). The application of S-carvone as a formulation in granules caused lower concentrations in leachates in comparison with the free compound (Figure 4). In addition, the peak of maximum concentration of S-carvone shifted to a larger volume of water added, yielding a more flattened curve compared to the free form (Figure 4). This flattening and tailing effect has typically been described for the leaching of pesticides when supported on other types of granules or organoclays [55]. Additionally, the total amounts leached for the free and the formulated compound were markedly different, 85 ± 2% and 36 ± 4%, respectively (Figure 4). These results confirmed that losses by the downward movement of S-carvone could be controlled by its formulation in granules, which is in line with the progressive release examined in the batch study conducted in water (Figure 2).

Once the leaching experiment ended, the soil inside the columns was extracted and analyzed. While no S-carvone remained in the soil when the allelochemical was applied as a free compound, 8 ± 3% of the allelochemical was recovered as soil residues when added as granules (Table 2). Thus, the slow release from the granules prolonged the presence of S-carvone as soil residues at moderate concentrations. The amount of allelochemical not recovered (Table 2) should have suffered degradation, volatilization, or, in the case of the formulation, could have also remained sorbed or trapped in the granules, which stayed at the sand surface layer of the columns at the end of the experiment. Nevertheless, most of the allelochemical that could have remained in the granules was, presumably, potentially releasable with time according to Figure 2.

Table 2. Data derived from the relative and cumulative breakthrough curves in the leaching experiment.

Treatment	Leached (%)	Extracted (%)	Not Recovered (%)	Position Cmax (Vp) [1]
S-carvone (Free)	85 ± 2	<1	15	1.5
S-carvone formulation	36 ± 4	8 ± 3	56	2.0

[1] Number of pore volumes of water at which the maximum concentration of S-carvone appeared in leachates.

3.4. Phytotoxicity of S-Carvone in Soil

The results of the bioassay conducted to determine the performance of the prepared granules to improve the phytotoxicity of S-carvone in the soil, as compared to the free compound, are summarized in Figure 5 and Figure S3. Data were compared according to aerial biomass, seed germination, and root length of *Lactuca sativa* L., stated as percentages of the control (without treatment with S-carvone). The effect of blanks with methanol and granules, but without S-carvone, were also compared with the control.

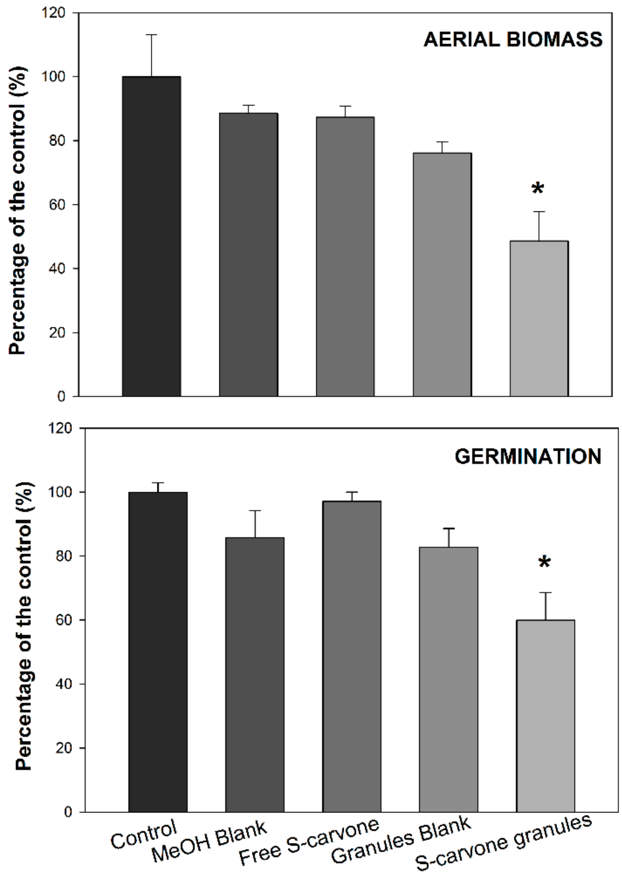

Figure 5. Effect of S-carvone applied as free compound or supported on granules on aerial biomass and germination of *Lactuca sativa* L. in the soil after 7 days of treatment. Bars are means and standard errors for $n = 3$. The asterisk indicates significant differences between the treatments and the control at statistical level $p < 0.05$.

The treatment that displayed the highest effect on the aerial biomass of *Lactuca sativa* L. was S-carvone in granules (Figure 5). S-carvone in free form did not provoke a significant effect, with the aerial biomass being similar to that observed for the control (Figure 5).

Additionally, neither the presence of methanol nor granules developed a significant impact on this parameter (Figure 5). Interestingly, seed germination was also significantly reduced for the granule formulation compared to the control and free S-carvone treatments, while the addition to soil of methanol or granules did not have significant effects (Figure 5). These results are congruent with the bioactivity described previously for monoterpenes, reporting that this family of compounds mainly affects seed germination [13,15,56–58]. Nevertheless, even though most related works focused on germination, Reynolds pointed out the importance of differentiating between seedling emergence and subsequent seedling growth [59], and, here, we found a significant reduction of these two parameters only for the granulated formulation of S-carvone (Figure 5).

Root length of emerged seedlings was similar for all treatments (Figure S3), even though other works have reported the effects of monoterpenes on root growth for certain plants [56,57]. Apart from the use of different target plant species, discrepancies could have also been a consequence of the specific conditions of the bioassays since soil has rarely been used as a medium growth in monoterpene bioassays [56]. Finally, considering that the degradation of S-carvone after being applied as a free compound or granules to the soil pots should have been similar (Figure 3) and that no leaching losses occurred during the bioassay, a plausible explanation for the superior herbicidal performance of the granulated formulation in the bioassay could have been a reduction in volatilization losses. Volatilization is known to be a primary source of losses for monoterpenes, and different methodologies for its reduction have previously been proposed, including the impregnation into polymers [27] or the encapsulation into biological microspheres [60]. We conducted a simple test to compare volatilization losses of S-carvone from the solution and the granulated formulation and found that, indeed, such losses were reduced for the formulated form of the allelochemical (Table S1). A reduction in volatilization losses could, thus, have accounted for the increased bioactivity of the granules that was apparent in the soil pot study (Figure 5).

4. Conclusions

Novel granules of the allelochemical S-carvone based on the commercial organoclay Cloisite® 10A were successfully prepared and the phytotoxicity of the organoclay-formulated S-carvone in soil was appraised for the first time. The granules displayed satisfactory features for the slow release of the allelochemical into water and were able to reduce transport losses without greatly affecting its degradation rate in soil, as compared with the free form. Most importantly, only by being supported on the granules, the monoterpene displayed its phytotoxic effect in soil, and no significant bioactivity was observed when applied as the free compound. The formulation was able to control the aerial biomass and seed germination of *Lactuca sativa* L. whereas the root growth was not affected. Our results indicate that S-carvone could be useful as a potential bioherbicide after being suitably stabilized in soils and may inspire new formulations for monoterpene-based bioherbicides. Further studies would be needed for the possible improvement of the proposed formulation through the optimization of variables such as the nature of the organoclay, the monoterpene loading, or the physical characteristics of the prepared granules. Likewise, investigations on the effect of these formulations on soil biodiversity and their performance under real field conditions would be convenient for the full comprehension of their environmental behavior and their possible commercialization.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11081593/s1, Figure S1: Photograph of powder and granules of Cloisite 10A®, Figure S2: Dissipation curves for S-carvone applied as a free compound to soil at an application dose of 2 mg/kg, Figure S3: Effect of S-carvone applied to soil as a free compound or supported on granules on root length of *Lactuca sativa* L., Table S1: Assessment of volatilization losses for the free and granulated forms of S-carvone.

Author Contributions: Conceptualization, B.G. and R.C.; methodology, B.G. and R.C.; formal analysis, B.G. and R.C.; investigation, B.G.; writing—original draft preparation, B.G.; writing—review and editing, B.G. and R.C.; visualization, B.G.; funding acquisition, R.C. Both authors have read and agreed to the published version of the manuscript.

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