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Abstract

Ailanthone, a quassinoid from *Ailanthus altissima* (Mill.) Swingle, is a natural herbicide, whose use is limited by its low persistence and rapid degradation in organic substrates. Dextrin-based nanosponges (NSs) are polymers with a cage-like structure that can complex several molecules, acting as carriers or protectors. Their encapsulation efficiency can be exploited in numerous applications. Hence this study explored at first the biological activity of eight different dextrin-based NSs, synthesized with 1,1'-carbonyldiimidazole (CDI) or pyromellitic dianhydride (PYRO) (αNS-CDI, βNS-CDI, γNS-CDI, LC NS-CDI, αNS-PYRO, βNS-PYRO, γNS-PYRO, and LC NS-PYRO), towards two model species (*Lepidium sativum* L. and *Raphanus sativus* L.) in filter paper under controlled conditions in laboratory. Then, the selected dextrin-based NSs were loaded with ailanthone and applied in the concentration of 7.5 or 30 mg L^{-1} of ailanthone in pre-emergence on the same species, initially on filter paper and subsequently on cultivation substrate for horticulture. In all three bioassays, the number of germinated seeds and the length of developed roots and hypocotyls were evaluated. In the first bioassay, the results showed that five dextrin-based NSs promoted the germination and root elongation, thus counteracting the herbicidal effect of ailanthone. Hence, three selected formulations (αNS-CDI, γNS-CDI, and LC NS-CDI) were loaded with ailanthone, with γNS-CDI providing the highest loading capacity (1.36%) and encapsulation efficiency (55.15%). In the second bioassay, the phytotoxic activity of ailanthone was strengthen by dextrin-based NSs, always stronger by at least 58% than the pure compound across 30 days in paper, without differences between formulations. In the third bioassay, loading ailanthone in γNS-CDI also prolonged its herbicidal activity, still reducing to only 20% the germination and growth of garden cress and radish 30 and 20 days after treatment, respectively. Overall, results demonstrated that dextrin-based nanosponges can be proposed as suitable carriers in the formulation of ailanthone-based herbicide. Their use both increased and extended the phytotoxic activity of ailanthone, leading to the possibility of reducing the amount applied for each treatment, or reducing the number of herbicide treatments.

Keywords: *Ailanthus altissima*, cyclodextrin, maltodextrin, phytotoxicity, pre-emergence, quassinoid

Abbreviations

Ail: ailanthone

Ail-NS-CDI: ailanthone and nanosponge complex

CD: cyclodextrin

CDI: 1,1’-carbonyldiimidazole

DAT: days after treatment

DMF: N,N-dimethylformamide

DMSO: dimethyl sulfoxide

IGe: index of germination

IGr: index of growth

LC: Kleptose Linecaps® Dextrose Equivalent 17

NS: nanosponge

NS-CDI: carbonate NS prepared from 1,1'-carbonyldiimidazole

NS-PYRO: ester NS prepared from pyromellitic dianhydride

PYRO: pyromellitic dianhydride

1. Introduction

Ailanthone (Ail) is a natural compound derived from *Ailanthus altissima* (Mill.) Swingle, a tree of Simaroubaceae family (Kowarik and Säumel, 2007). Ailanthone belongs to the quassinoids, natural compounds characterising Simaroubaceae plants, extensively studied for their antitumor, antimalarial, anti-inflammatory, antiparasitic, antifeedant, and herbicidal activities (Caser et al., 2020; Curcino Vieira and Braz-Felho, 2006; Daga et al., 2019; Dayan et al., 1999; Demasi et al., 2019a, 2019b). In particular, Ail phytotoxic activity was seen both in pre-emergence and post-emergence stage of some species, showing a non-selective spectrum of herbicidal effects on monocots and dicots under controlled conditions (Heisey and Heisey, 2003). Nevertheless, as well as other natural compounds, several constraints impede the commercial development of a natural herbicides based on Ail (Bhowmik and Inderjit, 2003; Duke et al., 2000; Heisey, 1999, 1996; Kowarik and Säumel, 2007; Sladonja et al., 2015; Soltys et al., 2013). The main problems are the high extraction and purification costs, as Ail is not currently produced on industrial scale, leading the cost of the pure compound between 2,000 and 3,000 Euros per gram, and the brief period of Ail efficacy in field soil, demonstrated in previous trials performed in greenhouse (Heisey, 1996, 1990) or field (Heisey and Heisey, 2003). Recent studies (Demasi et al., 2019a, 2019b) started to explore the feasibility and efficacy of Ail application in the horticulture sector, where much lower doses of herbicides are used if compared with the open field. Besides, Ail application for weed control in urban green areas has been suggested, since in this context human exposure to synthetic products and the related environmental issues are currently a matter of concerns in Europe (EU Regulation No 1107/2009 and Directive 2009/128/ECCE). Results (Demasi et al., 2019a, 2019b) confirmed a strong herbicidal activity of Ail on two model species (*Lepidium sativum* L. – garden cress, and *Raphanus sativus* L. – radish) already at low doses (7.5 mg L^{-1}) in paper in growth chamber, while moving to organic substrate for horticulture, higher doses (at least 30 mg L^{-1}) were necessary. In both cases, the effect persisted for 20-30 days.

Synthetic or natural herbicides can be combined with several carriers in order to increase or extend their efficacy and the proper formulation might markedly affect the phytotoxic effect of a compound (Duke, 2017). Complexing molecules in polymer matrices is an effective strategy to protect them from degradation and release them with controlled and prolonged kinetics (Conte et al., 2014; Lo Meo et al., 2014; Taban et al., 2020; Venuti et al., 2017). Dextrin-based nanosponges (NSs) are hyper-cross-linked polymers that could be used to this end, as they own a cage-like three-dimensional structure able to entrap several molecules. Cyclodextrins (CDs) and maltodextrins are the main components of this typology of NSs. Cyclodextrins are starch-derived cyclic oligosaccharides composed of glucopyranose units linked by α-1,4-glycosidic bonds. Alpha-, β-, and γ-CD, consisting of six, seven, and eight glucopyranose units arranged around a central cavity of approximately 0.57, 0.78, and 0.95 nm diameter, are the most widely used CDs (Bilensoy, 2011; Szejtli, 1988). Maltodextrins can act as complexing agents as well, thanks to the helical structure of their amylose chains. Among commercially available maltodextrins, Kleptose Linecaps® DE17 (LC – Roquette Frères, Lestreme, France) is a highly soluble product, prepared via partial hydrolysis of pea starch. The encapsulation properties of LC derive from its high content of amylose, which is nearly 40% (Boursier, 2009; Juluri et al., 2016). Suitable bi- or poly-functional reactants, such as dianhydrides, active carbonyl compounds, diglycidyl ethers, diisocyanates, etc. can crosslink dextrins to form NSs. Dextrin-based NSs are usually able to encapsulate a wider spectrum of molecules, if compared with native dextrins, since in NSs guest molecules can be accommodated in the internal volumes of dextrins as well as in the interstitial spaces among dextrins (Caldera et al., 2017; Trotta and Fossati, 2017). Thereof, dextrin-based NSs have several applications, from drug delivery (Allahyari et al., 2019; Massaro et al., 2016) to pollutants removal (Baglieri et al., 2013). In horticulture, CD-NSs have been used successfully in previous studies to extend the postharvest quality and longevity of cut flowers through the loading of 1-Methylcyclopropene (Seglie et al., 2013, 2012, 2011a, 2011b). They could serve as herbicide carrier as well (Pawar et al., 2019), however this application is almost unexplored (Liu et al., 2020).

In this study, carbonate and ester NSs were synthesized according to established and patented procedures (Allahyari et al., 2019; Ramírez-Ambrosi et al., 2014; Conte et al., 2014; Trotta and Fossati, 2017; Trotta and Tumiatti, 2003; Trotta et al., 2004) by crosslinking LC, α-, β-, and γ-CD with 1,1'-carbonyldiimidazole and pyromellitic dianhydride, respectively, to evaluate their biological activity on two model species (*L. sativum* and *R. sativus*) in pre-emergence in growth chamber, since their effects on plants were unknown. Then, the selected formulations were loaded with Ail and tested on the same species in filter paper and substrate for horticulture production. The study aimed at identifying for the first time a suitable formulation able to host Ail effectively and which possibly favours its efficacy, allowing to use smaller quantities of this compound.

2. Material and methods

2.1. Chemicals

α-CD and γ-CD were kindly provided by Wacker Chemie AG (Munich, Germany), while β-CD and LC by Roquette Freres (Lestrem, France). Cyclodextrins and LC were desiccated in oven at 80°C up to constant weight prior to use. Ailanthone was purchased from Herbest (Baoji Herbest Bio-Tech Co., Ltd. Baoji, China). All the other chemicals mentioned in this study were purchased from Sigma-Aldrich (Saint Louis, US) and used as received, with the exception of N,N-dimethylformamide (DMF), which was treated with calcium hydride for anhydrification and then filtered, before use.

2.2. Synthesis of dextrin-based nanosponges

2.2.1. Synthesis of carbonate NSs

Carbonate NSs (NS-CDI, Figure 1) were prepared by heating a solution of dextrin and 1,1'-carbonyldiimidazole (CDI) in anhydrous DMF (Trotta and Tumiatti, 2003). Precisely, 6.500 g of dextrin were dissolved in 39 mL of DMF. After the addition of the proper amount of CDI (Table 1), the solution was heated at 90°C for 4 h. The rigid gel, that was formed during the crosslinking reaction, was ground in a mortar and washed with deionized water through Buchner filtration. After rinsing with acetone, the NS was purified by Soxhlet extraction in acetone for approximately 24 h and finally left to dry at ambient temperature. The NS-CDI were prepared in a 1:4 dextrin/CDI molar ratio. Kleptose Linecaps® DE17 NS-CDI was synthesized using the same dextrin/CDI mass ratio of βNS-CDI, since LC has not a well-defined molecular weight.

2.2.2. Synthesis of pyromellitic NSs

A typology of ester NS, having pyromellitic bridges connecting dextrins (NS-PYRO, Figure 1), was synthesized by reacting dextrins with pyromellitic dianhydride (PYRO) in the presence of triethylamine (Et_3N) (Trotta et al., 2004). In details, 4.886 g of dextrin were solubilized in 20 mL of dimethyl sulfoxide (DMSO). Afterwards, 5 mL of triethylamine and the required amount of pyromellitic dianhydride (Table 2) were introduced under continuous stirring at room temperature. As a result of the crosslinking reaction, a rigid gel was formed in just a few minutes. Twenty-four hours later, the gel was crushed in a mortar and then washed in a Buchner funnel with a large amount of deionized water and finally rinsed with acetone. Further purification of the NS was carried out by Soxhlet extracting the NS in acetone for approximately 24 h. Analogously to NS-CDI, NS-PYRO were prepared in a 1:4 dextrin/PYRO molar ratio. Kleptose Linecaps® DE17 NS-PYRO was synthesized using the same dextrin/PYRO mass ratio of βNS-PYRO, since LC has not a well-defined molecular weight.

2.3. FT-IR characterization of dextrin-based nanosponges

All the synthesized NSs were characterized by means of Fourier Transform Infrared Spectroscopy in Attenuated Total Reflectance mode (FTIR-ATR) using a PerkinElmer Spectrum 100 spectrometer. The FTIR-ATR spectra were collected between 4000 and 650 cm^{-1} at a resolution of 4 cm^{-1} and scan number of 8.

2.4. Bioactivity of dextrin-based nanosponges

The bioactivity of the synthesized dextrin-based nanosponges was tested in the laboratories of the Department of Agriculture, Forest, and Food Sciences of the University of Torino in Italy (45°03′58.5″ Lat. N; 7°35′29.1″ Long. E). Trials were performed on two model species (garden cress – *L. sativum* 'Inglese' – and radish – *R. sativus* 'Tondo Rosso BIO'), which are fast-growing and are differently affected by toxins. Ten seeds per species were randomly put on one layer of filter paper (Whatman No. 1, Whatman, Maidstone, UK) in 90 mm Petri plates and spiked with 5 mL of treatment. Specifically, αNS-CDI, βNS-CDI, γNS-CDI, LC NS-CDI, αNS-PYRO, βNS-PYRO, γNS-PYRO, and LC NS-PYRO, were diluted with deionised water to obtain three different concentrations of each (10, 100 and 1000 mg L^{-1}). Deionised water was used as control treatment.

Three plates were prepared per treatment per species and the experiment was performed in triplicate, for a total of 90 seeds; plates were covered with their lid, but not sealed and kept in a growth chamber in the dark at 25°C for 96 hours (ISTA, 2011). Then, in treated (t) and control (c) plates, the number of germinated seeds (n) and mean root length (r) of developed seedlings were recorded to calculate the following Index of Germination (IGe%), according to Demasi et al., (2019b):

$$\text{IGe\%} = \frac{n_{(t)} * r_{(t)}}{n_{(c)} * r_{(c)}} * 100 \quad (1)$$

2.5. Inclusion of ailanthone in NSs and quantification of loaded ailanthone

According to the results of the dextrin-based nanosponges bioactivity, the β-CD NSs and the NSs prepared with PYRO as reactant were excluded from further trial. The encapsulation of Ail in αNS-CDI, γNS-CDI, and LC NS-CDI was achieved by stirring 2.000 g of NS in 10 mL of a 5 mg mL^{-1} solution of Ail in methanol for 24 h. Subsequently, the NSs were recovered by filtration, dried at room temperature and stored in hermetic vials at 2-8°C.

The amount of Ail loaded in the NSs was quantified by means of High-Performance Liquid Chromatography (HPLC) analysis. The extraction of Ail was accomplished by stirring 50 mg of NS in 2.5 mL of water:methanol (75:25 v:v) solution. Twenty-four hours later, the dispersion was centrifuged at 4,000 rpm for 10 min and the supernatant was recovered as first extract and then replaced with 2.5 mL of fresh water:methanol solution. The extraction was repeated five more times. All the extracts were filtered over 0.2 µm polytetrafluoroethylene (PTFE) syringe filters before injection. HPLC analysis was carried out at room temperature, using a PerkinElmer Brownlee Analytical C18 chromatographic column (250 mm x 4.6 mm, particle size 5 µm) connected to a PerkinElmer HPLC system, comprising a Flexar pump working at a flow rate of 1 mL min^{-1} and Flexar UV-VIS detector set at 254 nm. The mobile phase was prepared mixing water and methanol (75:25 v:v) and elution was isocratic. The total run time was set to 14 min, while the retention time of Ail was observed at 7 min, approximately. Ailanthone was quantified against an external calibration curve with standards (1, 2, 5, 10, 20, 50, 70, 100 μg mL^{-1}) prepared by serial dilution with mobile phase of a 1000 μg mL^{-1} stock solution. Loading capacity and encapsulation efficiency were calculated using the following equations:

$$Loading\ capacity\ (\%) = \frac{Ail\ extracted\ from\ the\ NS\ (mg)}{NS\ loaded\ with\ Ail\ (mg)} * 100 \qquad (2)$$

$$Encapsulation\ efficiency\ (\%) = \frac{Ail\ extracted\ from\ the\ NS\ (mg)}{Ail\ used\ for\ the\ loading\ (mg)} * 100 \qquad (3)$$

The loading capacity represents the percentage amount of Ail loaded in the NS, with respect to the weight of the NS, whereas the encapsulation efficiency expresses the fraction of Ail that the NS was able to absorb during the loading step.

2.6. Bioactivity of dextrin-based nanosponges loaded with ailanthone

The bioactivity of αNS-CDI, γNS-CDI, and LC NS-CDI loaded with Ail was evaluated on two model species (garden cress and radish) in a growth chamber at 25°C, with 12 h-photoperiod (55 μmol m^{-2} s^{-1} under cool, white fluorescent lamps). αNS-CDI, γNS-CDI, and LC NS-CDI loaded with Ail were diluted with deionised water to obtain the concentrations of 7.5 or 30 mg L^{-1} of Ail in the solution. These doses were previously seen to be effective for pure Ail in filter paper and substrate for horticulture, respectively (Demasi et al., 2019b). Five seeds per species were randomly placed on one layer of filter paper in 100 mL plastic flasks (base diameter 4.5 cm, top diameter 5.5 cm), suitable to allow seedling elongation. Seeds were sprinkled with 1.7 mL of the treatment or deionised water as control at the beginning of the trial (0 Days After Treatment – DAT). Flasks were covered with their lid, but not sealed. Six flasks (replicates) per treatment per species were prepared and the experiment was performed in triplicate, for a total of 90 seeds. The bioactivity of the formulations sprinkled at 0 DAT was evaluated at three time-points, i.e. 10, 20, and 30 DAT on renewed seeds, without treating anymore. In detail, at 10 DAT, the number of germinated seeds (n) and the root (r) and hypocotyl (h) length of developed seedlings were recorded in treated (t) and control (c) flasks to calculate the Index of Growth (IGr%) according to Demasi et al. (2019b):

$$IGr\% = \frac{n_{(t)} * r_{(t)} * h_{(t)}}{n_{(c)} * r_{(c)} * h_{(c)}} * 100 \qquad (4)$$

The evaluated seedlings and/or non-germinated seeds were removed with tweezers and new seeds were placed on the filter paper, solely adding 1.7 mL of deionised water to prevent dryness. The measurements to calculate IGr% were performed at 20 DAT; the procedure was repeated, acquiring data also at 30 DAT.

Analogously, the same trial was performed in a cultivation substrate for horticulture (Floradur® B Seed, Floragard Vertriebs-GmbH). Flasks were filled with 20 g of substrate and wetted with 5 mL of deionised water the day before the experiment. At 0 DAT, five seeds per species were randomly placed on the substrate and sprinkled with treatments (7.5 and 30 mg L^{-1} of Ail) or deionised water. Seedlings and/or non-germinated seeds were evaluated at three time-points (10, 20, and 30 DAT), on renewed seeds.

2.7. Statistical analyses

Arcsine transformation was made on IGe and IGr percentages prior to analysis; the reported values are means of untransformed data. Data were tested for the homogeneity of variance (Levene test) and one-way ANOVA was performed on IGe and IGr to compare the biological activity of dextrin-based nanosponges and formulations loaded with Ail, at different concentrations and time-points. The IGe% and IGr% of control plates and flasks were obtained using the average values as control data (c) and each repetition as treatment data (t) in Equations (1) and (4). Tukey post-hoc test ($p < 0.05$) was used to identify significant differences (SPSS Inc., V25, Chicago, Illinois).

3. Results

3.1. FT-IR characterization of dextrin-based nanosponges

The FTIR-ATR spectra of both PYRO and CDI NSs show a broad band between 3600 and 3000 cm^{-1}, due to O-H stretching vibrations and the typical absorption peaks of C-H stretching vibrations in the 3000-2850 cm^{-1} range, whereas the stretching vibrations of C-O bonds in alcohol and ether moieties appear at approximately 1240 and 1025 cm^{-1}, respectively. The presence of the crosslinker in the polymer structure of both PYRO and CDI NSs is confirmed by a strong absorption peak located at approximately 1700 cm^{-1} (1740 cm^{-1} in the case of CDI NSs, 1720 in PYRO NSs), which can be attributed to the stretching vibrations of carbonyl groups (not visible in the spectra of dextrins).

The NSs prepared with the same crosslinker, but different dextrin, exhibit the same absorption peaks, as they contain the same functional groups. FTIR-ATR analysis did not reveal the presence of ailanthone in the ailanthone-loaded NSs. This is probably due to the low content of ailanthone and the intense absorption peaks of the nanosponges covering the peaks of ailanthone. However, the quantification of ailanthone was successfully assessed by HPLC analysis, as described below.

3.2. Bioactivity of dextrin-based nanosponges

The eight CD-NSs synthesized were tested on garden cress and radish without loading the Ail to test their biological activity. Data showed that the treatments differently stimulated the germination and root growth of the two model species compared with water control (Table 3). In the first species, all the dextrin types slightly promoted the IGe, but the β-cyclodextrin scored the highest value (116.8%), while no differences were attributable to the reactant, which IGe values were similar to the control, whether PYRO or CDI. Conversely, in the second species, no differences between dextrin types were recorded, while the PYRO reactant highly stimulated seeds germination and root length, giving an IGe higher than control (120%).

3.3. Quantification of loaded ailanthone

Repeated extractions in water-methanol mixture were performed in order to evaluate the Ail content of the ailanthone-loaded NSs. The mass percentage of Ail, extracted from the NSs, is cumulatively plotted against time in Figure 2. For all tested NSs, three extractions were enough to remove the entire amount of Ail. A total amount of Ail equal to 0.92%, 1.36%, and 1.16% was extracted from Ail-αNS-CDI, Ail-γNS-CDI, and Ail-LC NS-CDI, respectively. Being calculated according to Eq. (1), these values also represent the loading capacities of the above listed NSs. As it appears from Table 4, loading capacity and encapsulation efficiency increase with the size of the dextrin cavity (from approximately 0.57 to 0.95 nm diameter from αNS-CDI to γNS-CDI), ~~with~~ reaching the maximum values in the case of Ail-γNS-CDI (1.36% of loading capacity and 55.15% of encapsulation efficacy). The cavity of αNS-CDI, not large enough to form an inclusion complex with Ail, could probably host one of its hydrophobic moieties, while the rest of the molecule was encapsulated in the interstitial space between CDs (secondary cavities). This speculation seems to be confirmed by the amount of Ail encapsulated in αNS-CDI (0.92 %), not negligible despite lower than γNS-CDI (1.36 %).

3.4. Bioactivity of dextrin-based nanosponges loaded with ailanthone

In filter paper, Ail-αNS-CDI, Ail-γNS-CDI, and Ail-LC NS-CDI were extremely phytotoxic compared with water control both at 7.5 and 30 mg L^{-1} in each day of evaluation (10, 20, and 30 DAT) and across time, without showing statistical differences between formulations and concentrations. Indeed, the IGr% values ranged from 0% to 0.45% in garden cress (Table 5) and they were always equal to 0% in radish (Table 6).

In the substrate for horticulture, extremely low IGr% were recorded compared with water control at 10 DAT both in garden cress (0-3%, Table 7) and radish (0-0.48%, Table 8), showing no significant differences between Ail-αNS-CDI, Ail-γNS-CDI, and Ail-LC NS-CDI, and 7.5 or 30 mg L^{-1}. Afterwards, at 20 DAT, the IGr% was significantly higher than IGr% at 10 DAT in treated seeds of garden cress, ranging from 74% to 108% and to a lesser extent, also in radish, ranging from 43% to 78%. However, at this time-point, the formulation of γNS-CDI at 30 mg L^{-1} of Ail still reduced the IGr to circa 20% in both model species, showing an improved phytotoxic activity than the other formulations and control. The herbicidal effect was almost completely lost at 30 DAT in both species (IGr=75-96% in garden cress and IGr=96-140% in radish), with no differences between the control, the formulation used, and the concentration applied.

4. Discussion

The results of the present study confirm Ail strong phytotoxicity towards two model species, namely garden cress and radish, previously recorded by different studies (Caser et al., 2020; Demasi et al., 2019a, 2019b; Heisey, 1996, 1990; Heisey and Heisey, 2003). However, Ail is expensive, has a short persistence in the environment, as observed in other natural compounds (Sladonja et al., 2015), and is subjected to a first-order degradation kinetic in organic substrates (Demasi et al., 2019b). In this study, dextrin-based NSs were evaluated for the first time as potential carriers for Ail, studying a suitable formulation for its application that possibly strengthen (i.e. increase the efficacy) or lengthen (i.e. prolong the efficacy) its herbicidal activity.

At first, all the cyclodextrins and maltodextrins were tested to evaluate their biological activity. The tested formulations somewhat promoted the growth of model species in paper, without loading Ail. The β-CD NSs and the NSs prepared with PYRO as reactant gave significantly higher IGe compared with control in garden cress and radish. Thus, these formulations that most promoted IGe in the model species were not loaded with Ail and were excluded from the successive trials to avoid a growth enhancement that could have counteracted the herbicidal purpose of Ail application. In literature, studies performed on the effect of CD-NSs on plants are almost lacking, but no significant differences in the growth of sweet corn was reported after the application of CD-NS loaded with iron (Fe) in hydroponics, compared with $FeSO_4$ and Fe-DTPA (Vercelli et al., 2015).

In the PYRO and CDI NSs, hydrolysis usually occurs in a few weeks or months, respectively. The result of this process is the complete degradation of the polymer into soluble fractions, composed of oligomers and the starting monomers, which can be easily absorbed by the plants, thus possibly acting as fertilizers. The effect of CD-NSs *per se* on seeds and plants should be therefore further investigated.

The selected formulations (αNS-CDI, γNS-CDI, and LC NS-CDI) were then loaded with Ail and all were suitable to host this molecule, though with different loading capacity and encapsulation efficiency. More specifically, the γ-CD-based NS seems to have the highest affinity for Ail, as the fraction of Ail that is retained after the first extraction is higher, if compared with the other NSs (Figure 2). The loading capacity values listed in Table 4 are comparable to those presented in previous studies. Peila et al. (2017) used NSs based on β-CD and CDI to store and release the insect-repellent N,N-diethyl-meta-toluamide (DEET) with slow kinetics. The amount of DEET that the NSs were able to encapsulate is between 0.5 and 2 wt%, approximately. While, in a study by Ramírez-Ambrosi et al. (2014) slightly higher values of loading capacity (1.9-3.2 wt%) were achieved by encapsulating polyphenols (i.e. phloridzin, rutin, and chlorogenic acid) from apple in CDI-based CD NSs. As for the encapsulation efficiency, the results shown in Table 4 are in the range of values that were achieved in the two above-mentioned studies. Moreover, in addition to their complexing properties and negligible toxicity, the studied NSs offer the advantage of being hydrolysable in the presence of water (Caldera et al., 2017; Shende et al., 2015).

Considering the herbicidal trials, the application of the selected NS-CDI loaded with Ail showed remarkable results on cress and radish using filter paper as substrate, regardless the concentration. In Figure 3, these results have been compared with that obtained applying pure Ail in the same experimental conditions (Demasi et al., 2019b). At 10 DAT, Ail was most effective on garden cress when loaded in αNS-CDI, γNS-CDI, and LC NS-CDI, with an improved efficacy of 66.7%, 58.3%, and 66.7% respectively (Figure 3A) compared with the pure compound. Similarly, the herbicidal effect of loaded Ail was more intense than pure Ail also at 20 DAT and even more at 30 DAT, where the efficacy was 100% higher and zero seeds germinated. Concerning radish (Figure 3B), at 10 DAT no differences were recorded due to the already strong effect of pure Ail; anyway, the efficacy was improved by all dextrin-based nanosponges in the following evaluations (20 and 30 DAT), improving the herbicidal effect by 100%.

When moving to the horticulture substrate, all the treatments were highly effective towards both species compared with control at 10 DAT, when the IGr of both species was lower than 0.1%. Later (20 DAT) a lower phytotoxicity was recorded, being Ail-γNS-CDI at 30 mg L^{-1} the only treatment to still reduce the IGr to 20% in new sown seeds of garden cress (Figure 4A) and radish (Figure 4B) compared with the pure Ail (-70.6% in garden cress and -51.4% in radish). At 30 DAT, again γNS-CDI performed better than pure Ail in garden cress, with an improved effect of 38.7%, while this effect was lost in radish. The generally much higher IGr values at 20 and 30 DAT than that recorded in filter paper at the same time-points could have been probably caused either by the buffer capacity of the organic substrate used in the experiment, or a rapid degradation of Ail in this substrate (Demasi et al., 2019b).

These results outlined the ability of the tested formulations to effectively carry Ail and both strengthen and lengthen its phytotoxic activity. In particular, dextrin-based NSs increased Ail efficacy on both species at each time point in filter paper, allowing to reduce the amount of Ail applied. In cultivation substrate for horticulture, Ail activity was prolonged to 30 DAT in garden cress and 20 DAT in radish when applied in pre-emergence, with γNS-CDI being the most efficient formulation.

Conclusions

The outcomes of this study highlighted for the first time the aptitude of NSs to preserve the efficacy of Ail over time and to release it with prolonged kinetics, especially in paper, without showing differences between formulations in both model species, namely garden cress and radish. The efficacy was promoted also in cultivation substrate for horticulture, though to a lesser extent, where Ail-γNS-CDI performed better than pure Ail and the other formulations until 30 DAT in garden cress and 20 DAT in radish. This may result from a higher affinity of Ail for the larger cavity of γ-CD. Its extraction profile, loading capacity, and the encapsulation efficacy indeed suggested stronger physical interactions between Ail molecules and the cavities of γNS-CDI. Hence, dextrin-based nanosponges and γNS-CDI, in particular, can be suggested as suitable carriers in the formulation of Ail-based herbicide, being able to improve its phytotoxicity and persistence in laboratory under controlled conditions. These results suggest that fewer applications of herbicide can be performed or lesser amount of Ail can be used to obtain the same phytotoxic effects when loaded in dextrin-based nanosponges.

Author Contributions

Sonia Demasi: conceptualization, data curation, investigation, formal analysis, writing—original draft. **Matteo Caser**: conceptualization, data curation, investigation, formal analysis, writing—review and editing. **Fabrizio Caldera**: investigation, formal analysis. **Nilesh Kumar Dhakar**: investigation. **Francesco Vidotto**: conceptualization. **Francesco Trotta**: resources, supervision, validation. **Valentina Scariot**: conceptualization, resources, supervision, validation, writing—review and editing, project administration, funding acquisition.

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References

Allahyari, S., Trotta, F., Valizadeh, H., Jelvehgari, M., Zakeri-Milani, P., 2019. Cyclodextrin-based nanosponges as promising carriers for active agents. Expert Opin. Drug Deliv. https://doi.org/10.1080/17425247.2019.1591365

Baglieri, A., Nègre, M., Trotta, F., Bracco, P., Gennari, M., 2013. Organo-clays and nanosponges for acquifer bioremediation: Adsorption and degradation of triclopyr. J. Environ. Sci. Heal. Part B 48, 784–792. https://doi.org/10.1080/03601234.2013.780943

Bhowmik, P.C., Inderjit, 2003. Challenges and opportunities in implementing allelopathy for natural weed management. Crop Prot. 22, 661–671. https://doi.org/10.1016/S0261-2194(02)00242-9

Bilensoy, E., 2011. Cyclodextrins in Pharmaceutics, Cosmetics, and Biomedicine: Current and Future Industrial Applications, Cyclodextrins in Pharmaceutics, Cosmetics, and Biomedicine: Current and Future Industrial Applications. John Wiley & Sons, Inc., Hoboken, NJ, USA. https://doi.org/10.1002/9780470926819

Boursier, B., 2009. Use of maltodextrin and/or of pea glucose syrup for encapsulating hydrophobic compounds. WO/2009/024690.

Caldera, F., Tannous, M., Cavalli, R., Zanetti, M., Trotta, F., 2017. Evolution of cyclodextrin nanosponges. Int. J. Pharm. 531, 470–479. https://doi.org/10.1016/j.ijpharm.2017.06.072

Caser, M., Demasi, S., Caldera, F., Kumar Dhakar, N., Trotta, F., Scariot, V., 2020. Activity of *Ailanthus altissima* (Mill.) Swingle Extract as a Potential Bioherbicide for Sustainable Weed Management in Horticulture. Agron. 2020, Vol. 10, Page 965 10, 965. https://doi.org/10.3390/agronomy10070965

Conte, C., Caldera, F., Catanzano, O., D’Angelo, I., Ungaro, F., Miro, A., Pellosi, D.S., Trotta, F., Quaglia, F., 2014. β-cyclodextrin nanosponges as multifunctional ingredient in water-containing semisolid formulations for skin delivery. J. Pharm. Sci. 103, 3941–3949. https://doi.org/10.1002/jps.24203

Curcino Vieira, I.J., Braz-Felho, R., 2006. Quassinoids: Structural diversity, biological activity and synthetic studies. Stud. Nat. Prod. Chem. 33, 433–492. https://doi.org/10.1016/S1572-5995(06)80032-3

Daga, M., Pizzimenti, S., Dianzani, C., Cucci, M.A., Cavalli, R., Grattarola, M., Ferrara, B., Scariot, V., Trotta, F., Barrera, G., 2019. Ailanthone inhibits cell growth and migration of cisplatin resistant bladder cancer cells through down-regulation of Nrf2, YAP, and c-Myc expression. Phytomedicine 56, 156–164. https://doi.org/10.1016/j.phymed.2018.10.034

Dayan, F.E., Watson, S.B., Galindo, J.C.G., Hernández, A., Dou, J., McChesney, J.D., Duke, S.O., 1999. Phytotoxicity of quassinoids: Physiological responses and structural requirements. Pestic. Biochem. Physiol. 65, 15–24. https://doi.org/10.1006/pest.1999.2432

Demasi, S., Caser, M., Fogliatto, S., Vidotto, F., Trotta, F., Scariot, V., 2019a. Ailanthone inhibition data on seed germination and seedling growth of *Lepidium sativum* L. and *Raphanus sativus* L. Data Br. 26, 104550. https://doi.org/10.1016/j.dib.2019.104550

Demasi, S., Caser, M., Vanara, F., Fogliatto, S., Vidotto, F., Negre, M., Trotta, F., Scariot, V., 2019b. Ailanthone from *Ailanthus altissima* (Mill.) Swingle as potential natural herbicide. Sci. Hortic. (Amsterdam). 257, 108702. https://doi.org/10.1016/j.scienta.2019.108702

Duke, S.O., 2017. Pesticide Dose – A Parameter with Many Implications, in: ACS Symposium Series - Pesticide Dose: Effects on the Environment and Target and Non-Target Organisms. ACS Publications, pp. 1–13. https://doi.org/10.1021/bk-2017-1249.ch001

Duke, S.O., Dayan, F.E., Romagni, J.G., Rimando, A.M., 2000. Natural products as sources of herbicides: Current status and future trends. Weed Res. 40, 99–111. https://doi.org/10.1046/j.1365-3180.2000.00161.x

Heisey, R.M., 1999. Development of an allelopathic compound from tree-of-heaven (*Ailanthus altissima*) as a natural product herbicide, in: Cutler, J.S., Cutler, H.G. (Eds.), Biologically Active Natural Products: Agrochemicals. CRC Press, Boca Raton, pp. 57–68. https://doi.org/10.1201/9781420048629-8

Heisey, R.M., 1996. Identification of an allelopathic compound from *Ailanthus altissima* (Simaroubaceae) and characterization of its herbicidal activity. Am. J. Bot. 83, 192–200. https://doi.org/10.2307/2445938

Heisey, R.M., 1990. Alleopathic and herbicidal effects of extracts from tree of heaven (*Ailanthus altissima*). Am. J. Bot. 77, 662–670. https://doi.org/10.1002/j.1537-2197.1990.tb14451.x

Heisey, R.M., Heisey, T.K., 2003. Herbicidal effects under field conditions of *Ailanthus altissima* bark extract, which contains ailanthone. Plant Soil 256, 85–99. https://doi.org/10.1023/A:1026209614161

ISTA, 2011. International Seed Testing Association.

Juluri, A., Popescu, C., Zhou, L., Murthy, R.N., Gowda, V.K., P, C.K., Pimparade, M.B., Repka, M.A., Murthy, S.N., 2016. Taste masking of griseofulvin and caffeine anhydrous using Kleptose Linecaps DE17 by hot melt extrusion. AAPS PharmSciTech 17, 99–105. https://doi.org/10.1208/s12249-015-0374-1

Kowarik, I., Säumel, I., 2007. Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle. Perspect. Plant Ecol. Evol. Syst. 8, 207–237. https://doi.org/10.1016/j.ppees.2007.03.002

Liu, X., Li, W., Xuan, G., 2020. Preparation and characterization of β-cyclodextrin nanosponges and study on enhancing the solubility of insoluble nicosulfuron, in: IOP Conference Series: Materials Science and Engineering. Institute of Physics Publishing, p. 012108. https://doi.org/10.1088/1757-899X/774/1/012108

Lo Meo, P., Lazzara, G., Liotta, L., Riela, S., Noto, R., 2014. Cyclodextrin-calixarene co-polymers as a new class of nanosponges. Polym. Chem. 5, 4499–4510. https://doi.org/10.1039/c4py00325j

Massaro, M., Cinà, V., Labbozzetta, M., Lazzara, G., Lo Meo, P., Poma, P., Riela, S., Noto, R., 2016. Chemical and pharmaceutical evaluation of the relationship between triazole linkers and pore size on cyclodextrin–calixarene nanosponges used as carriers for natural drugs. RSC Adv. 6, 50858–50866. https://doi.org/10.1039/C6RA06143E

Pawar, S., Shende, P., Trotta, F., 2019. Diversity of β-cyclodextrin-based nanosponges for transformation of actives. Int. J. Pharm. 565, 333–350. https://doi.org/10.1016/j.ijpharm.2019.05.015

Peila, R., Scordino, P., Shanko, D.B., Caldera, F., Trotta, F., Ferri, A., 2017. Synthesis and characterization of β-cyclodextrin nanosponges for N,N-diethyl-meta-toluamide complexation and their application on polyester fabrics. React. Funct. Polym. 119, 87–94. https://doi.org/10.1016/j.reactfunctpolym.2017.08.008

Ramírez-Ambrosi, M., Caldera, F., Trotta, F., Berrueta, L., Gallo, B., 2014. Encapsulation of apple polyphenols in β-CD nanosponges. J. Incl. Phenom. Macrocycl. Chem. 80, 85–92. https://doi.org/10.1007/s10847-014-0393-7

Seglie, L., Devecchi, M., Trotta, F., Scariot, V., 2013. β-Cyclodextrin-based nanosponges improve 1-MCP efficacy in extending the postharvest quality of cut flowers. Sci. Hortic. (Amsterdam). 159, 162–165. https://doi.org/10.1016/j.scienta.2013.05.019

Seglie, L., Martina, K., Devecchi, M., Roggero, C., Trotta, F., Scariot, V., 2011a. β-Cyclodextrin-based nanosponges as carriers for 1-MCP in extending the postharvest longevity of carnation cut flowers: An evaluation of different degrees of cross-linking. Plant Growth Regul. 65, 505–

511. https://doi.org/10.1007/s10725-011-9621-y

Seglie, L., Martina, K., Devecchi, M., Roggero, C., Trotta, F., Scariot, V., 2011b. The effects of 1-MCP in cyclodextrin-based nanosponges to improve the vase life of *Dianthus caryophyllus* cut flowers. Postharvest Biol. Technol. 59, 200–205. https://doi.org/10.1016/j.postharvbio.2010.08.012

Seglie, L., Spadaro, D., Trotta, F., Devecchi, M., Gullino, M.L., Scariot, V., 2012. Use of 1-methylcylopropene in cyclodextrin-based nanosponges to control grey mould caused by *Botrytis cinerea* on *Dianthus caryophyllus* cut flowers. Postharvest Biol. Technol. 64, 55–57. https://doi.org/10.1016/j.postharvbio.2011.09.014

Shende, P., Kulkarni, Y.A., Gaud, R.S., Deshmukh, K., Cavalli, R., Trotta, F., Caldera, F., 2015. Acute and repeated dose toxicity studies of different β-cyclodextrin-based nanosponge formulations. J. Pharm. Sci. 104, 1856–1863. https://doi.org/10.1002/jps.24416

Sladonja, B., Sušek, M., Guillermic, J., 2015. Review on invasive tree of heaven (*Ailanthus altissima* (Mill.) Swingle) conflicting values: assessment of its ecosystem services and potential biological threat. Environ. Manage. 56, 1009–1034. https://doi.org/10.1007/s00267-015-0546-5

Soltys, D., Krasuska, U., Bogatek, R., Gniazdowsk, A., 2013. Allelochemicals as Bioherbicides — Present and Perspectives, in: Herbicides - Current Research and Case Studies in Use. InTech, pp. 517–542. https://doi.org/10.5772/56185

Szejtli, J., 1988. Cyclodextrin Technology, Topics in Inclusion Science. Springer Netherlands, Dordrecht. https://doi.org/10.1007/978-94-015-7797-7

Taban, A., Saharkhiz, M.J., Khorram, M., 2020. Formulation and assessment of nano-encapsulated bioherbicides based on biopolymers and essential oil. Ind. Crops Prod. 149, 112348. https://doi.org/10.1016/j.indcrop.2020.112348

Trotta, F., Fossati, E., 2017. A polymer based on a maltodextrin for encapsulating organic compounds. PCT/EP2014/064466.

Trotta, F., Tumiatti, W., 2003. Cross-linked polymers based on cyclodextrins for removing polluting agents. WO/2003/085002.

Trotta, F., Tumiatti, W., Vallero, R., 2004. Nanospugne a base di ciclodestrine funzionalizzate con gruppi carbossilici: sintesi e utilizzo nella decontaminazione da metalli pesanti e da composti organici. MI2004A000614.

Venuti, V., Rossi, B., Mele, A., Melone, L., Punta, C., Majolino, D., Masciovecchio, C., Caldera, F., Trotta, F., 2017. Tuning structural parameters for the optimization of drug delivery performance of cyclodextrin-based nanosponges. Expert Opin. Drug Deliv. 14, 331–340. https://doi.org/10.1080/17425247.2016.1215301

Vercelli, M., Gaino, W., Contartese, V., Gallo, L., Carlo, S. Di, Tumiatti, V., Larcher, F., Scariot, V., 2015. Preliminary studies on the effect of Fe-nanosponge complex in horticulture. Acta Sci. Pol. Hortorum Cultus 14, 51–58.

Tables

Table 1. Quantities of chemicals used for the synthesis of carbonate nanosponges ~~(NS). DMF=N,N-dimethylformamide; CDI=1,1'-carbonyldiimidazole~~.

	DMF[x]	Dextrin	CDI[y]	Molar ratio CDI/dextrin
	mL	g	g	
αNS-CDI	39	6.500	4.334	4
βNS-CDI	39	6.500	3.715	4
γNS-CDI	39	6.500	3.250	4
LC NS-CDI	20	6.500	3.715	-

[x]DMF=N,N-dimethylformamide;

[y]CDI=1,1'-carbonyldiimidazole

Table 2. Quantities of chemicals used for the synthesis of pyromellitic NSs. DMSO=dimethyl sulfoxide; Et_3N=triethylamine; PYRO=pyromellitic dianhydride.

	DMSO [x]	Dextrin	Et_3N [y]	PYRO [z]	Molar ratio PYRO/dextrin
	mL	g	mL	g	
αNS-PYRO	20	4.886	5	4.382	4
βNS-PYRO	20	4.886	5	3.756	4
γNS-PYRO	20	4.886	5	3.286	4
LC NS-PYRO	20	4.886	5	3.756	-

[x] DMSO=dimethyl sulfoxide

[y] Et_3N=triethylamine;

[z] PYRO=pyromellitic dianhydride

Table 3. Effects of dextrin type and reactant used to prepare the dextrin-based nanosponges on the index of germination (IGe~~%~~) of garden cress (*Lepidium sativum*) and radish (*Raphanus sativus*) in filter paper. Data are means ± standard error.

Species	Dextrin type	IGe %		Reactant	IGe %	
Garden cress	Control	100.1 ± 5.31	b[x]	Control	100.1 ± 5.31	
	α	108.3 ± 3.47	ab	PYRO	112.7 ± 1.93	
	β	116.8 ± 4.39	a	CDI	108.7 ± 2.07	
	γ	108.4 ± 2.76	ab			
	LC	109.2 ± 3.00	ab			
	p[y]	*			ns	
Radish	Control	100.0 ± 14.30		Control	100 ± 14.30	b
	α	113.5 ± 2.69		PYRO	120.0 ± 1.93	a
	β	119.9 ± 2.99		CDI	111.8 ± 2.07	b
	γ	116.3 ± 4.31				
	LC	114.0 ± 3.01				
	p	ns			*	

[x]Similar letters inside the same column denote no significant differences according to Tukey post-hoc test.

[y]The statistical relevance is provided (* = $p \leq 0.05$; ns = not significant).

Table 4. Loading capacity and encapsulation efficiency values of the carbonate NSs loaded with ailanthone (Ail).

Sample	Loading capacity %	Encapsulation efficiency %
Ail-αNS-CDI	0.92	37.14
Ail-γNS-CDI	1.36	55.15
Ail-LC NS-CDI	1.16	46.94

Table 5. Index of growth ~~(IGr%)~~ of garden cress (*Lepidium sativum*) in response to the application of water (control) or α, γ and LC NS-CDI loaded with ailanthone ~~(Ail)~~ to provide the dose of 7.5 and 30 mg L^{-1} of Ail in the solution. Data were obtained in filter paper, at 10, 20 and 30 days after treatment ~~(DAT)~~. Data are means ± standard error.

Formulation	Ail concentration mg L^{-1}	10 DAT[x]		20 DAT		30 DAT		p^y
Control	0	101.20 ± 10.21	a[z]	99.11 ± 1.74	a	101.65 ± 15.97	a	ns
Ail-αNS-CDI	7.5	0.36 ± 0.06	b	0.36 ± 0.23	b	0.00 ± 0.00	b	ns
	30	0.05 ± 0.05	b	0.00 ± 0.00	b	0.00 ± 0.00	b	ns
Ail-γNS-CDI	7.5	0.45 ± 0.27	b	0.20 ± 0.01	b	0.00 ± 0.00	b	ns
	30	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	ns
Ail-LC NS-CDI	7.5	0.41 ± 0.25	b	0.00 ± 0.00	b	0.00 ± 0.00	b	ns
	30	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	ns
p		***		***		***		

[x]DAT=days after treatment

[y]The statistical relevance is provided (*** = $p \leq 0.001$; ns = not significant).

[z]Similar letters inside the same column denote no significant differences according to Tukey post-hoc test.

Table 6. Index of growth ~~(IGr%)~~ of radish (*Raphanus sativus*) in response to the application of water (control) or α, γ and LC NS-CDI loaded with ailanthone ~~(Ail)~~ to provide the dose of 7.5 and 30 mg L^{-1} of Ail in the solution. Data were obtained in filter paper, at 10, 20 and 30 days after treatment ~~(DAT)~~. Data are means ± standard error.

Formulation	Ail concentration mg L^{-1}	10 DAT[x]		20 DAT		30 DAT		p[y]
Control	0	103.81 ± 26.84	a[z]	99.65 ± 14.21	a	110.44 ± 23.87	a	ns
Ail-αNS-CDI	7.5	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	ns
	30	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	ns
Ail-γNS-CDI	7.5	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	ns
	30	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	ns
Ail-LC NS-CDI	7.5	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	ns
	30	0.00 ± 0.00	b	0.00 ± 0.00	b	0.00 ± 0.00	b	ns
p		***		***		***		

[x]DAT=days after treatment

[y]The statistical relevance is provided (*** $p \leq 0.001$; ns = not significant).

[z]Similar letters inside the same column denote no significant differences according to Tukey post-hoc test.

Table 7. Index of growth ~~(IGr%)~~ of garden cress (*Lepidium sativum*) in response to the application of water (control) or α, γ and LC NS-CDI loaded with ailanthone (Ail) to provide the dose of 7.5 and 30 mg L^{-1} of Ail in the solution. Data were obtained in cultivation substrate for horticulture, at 10, 20 and 30 days after treatment ~~(DAT)~~. Data are means ± standard error.

Formulation	Ail concentration mg L^{-1}	10 DAT[x]		20 DAT		30 DAT		p[y]
Control	0	99.90 ± 4.32	a[z]	99.52 ± 2.40	a	99.69 ± 3.38		ns
Ail-αNS-CDI	7.5	3.00 ± 0.43	b B	91.33 ± 6.66	a A	93.28 ± 5.72	A	***
	30	0.09 ± 0.04	b B	74.11 ± 15.33	a A	85.87 ± 6.92	A	***
Ail-γNS-CDI	7.5	2.92 ± 0.71	b B	79.23 ± 9.94	a A	80.65 ± 5.31	A	***
	30	0.04 ± 0.03	b B	21.65 ± 9.17	b B	74.72 ± 10.31	A	***
Ail-LCNS-CDI	7.5	2.96 ± 0.58	b B	102.39 ± 8.86	a A	89.13 ± 7.88	A	***
	30	0.00 ± 0.00	b B	108.23 ± 14.33	a A	95.58 ± 6.62	A	***
p		***		***		ns		

[x]DAT=days after treatment

[y]The statistical relevance is provided (*** $p \leq 0.001$; ns = not significant)

[z]Similar upper-case letters along the same treatment and similar lower-case letters within the same DAT denote no significant differences according to the Tukey post-hoc test.

Table 8. Index of growth ~~(IGr%)~~ of radish (*Raphanus sativus*) in response to the application of water (control) or α, γ and LC NS-CDI loaded with ailanthone ~~(Ail)~~ to provide the dose of 7.5 and 30 mg L^{-1} of Ail in the solution. Data were obtained in cultivation substrate for horticulture, at 10, 20 and 30 days after treatment ~~(DAT)~~. Data are means ± standard error.

Formulation	Ail concentration mg L^{-1}	10 DAT[x]		20 DAT		30 DAT		p^y
Control	0	99.06 ± 6.68	a[z]	99.00 ± 2.41	a	99.80 ± 11.55		ns
Ail-αNS-CDI	7.5	0.48 ± 0.19	b B	64.50 ± 9.60	a A	113.79 ± 7.99	A	***
	30	0.00 ± 0.00	b B	61.87 ± 14.42	a A	121.02 ± 10.85	A	***
Ail-γNS-CDI	7.5	0.26 ± 0.06	b B	53.90 ± 3.41	a A	107.03 ± 8.35	A	***
	30	0.00 ± 0.00	b B	19.31 ± 8.88	b B	96.34 ± 13.58	A	***
Ail-LCNS-CDI	7.5	0.08 ± 0.03	b B	78.37 ± 11.79	a A	104.21 ± 20.80	A	***
	30	0.00 ± 0.00	b B	43.15 ± 12.13	a A	140.32 ± 20.41	A	***
p		***		***		ns		

[x]DAT=days after treatment

[y]The statistical relevance is provided (*** $p \leq 0.001$; ns = not significant)

[z]Similar upper-case letters along the same treatment and similar lower-case letters within the same DAT denote no significant differences according to the Tukey post-hoc test.

Figure 1

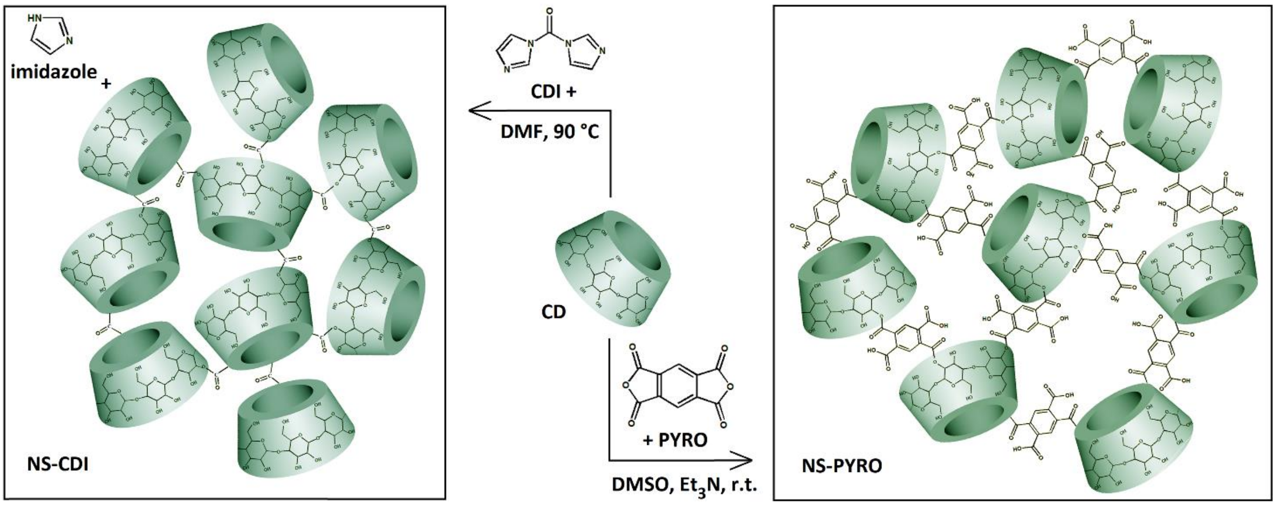

Figure 2

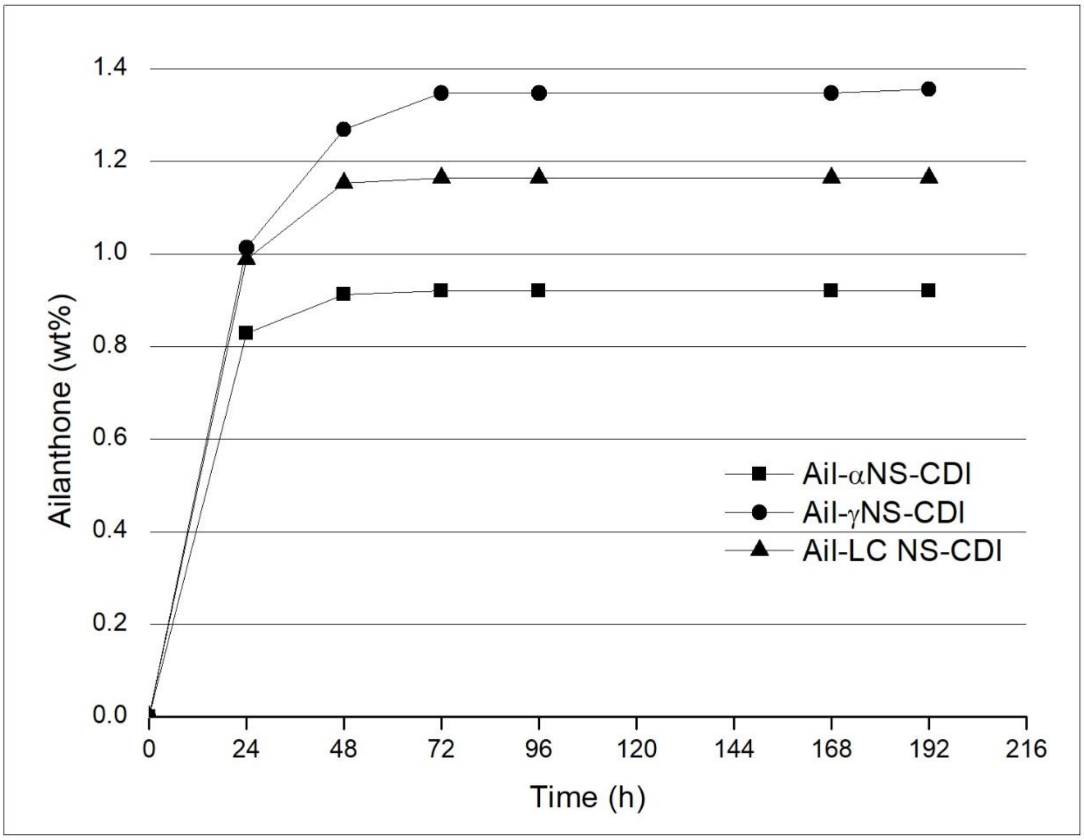
Ailanthone (wt%)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
24
48
72
96
120
144
168
192
216
Time (h)
Ail-αNS-CDI
Ail-γNS-CDI
Ail-LC NS-CDI

Figure 3

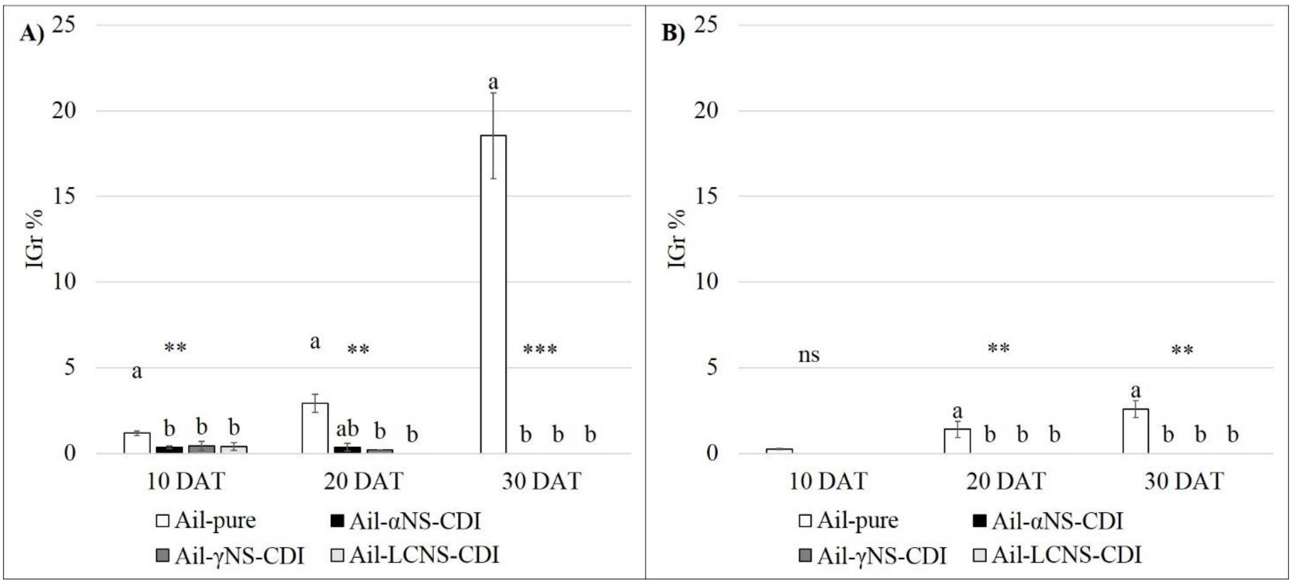
A)
B)
IGr %
25
20
15
10
5
0
10 DAT
20 DAT
30 DAT
a
b
ab
ns
**
***
Ail-pure
Ail-αNS-CDI
Ail-γNS-CDI
Ail-LCNS-CDI

Figure 4

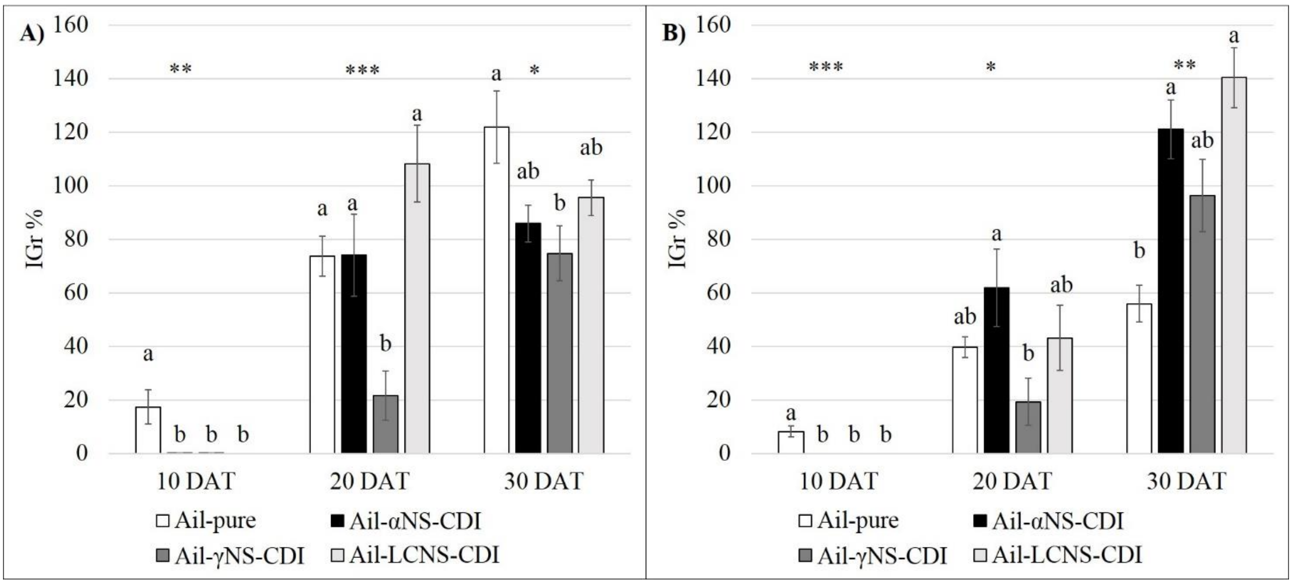
A)
B)
IGr %
160
140
120
100
80
60
40
20
0
10 DAT
20 DAT
30 DAT
Ail-pure
Ail-αNS-CDI
Ail-γNS-CDI
Ail-LCNS-CDI

Figure captions

Figure 1. Schematic representation of the synthesis reaction and crosslinked structure of the carbonate (on the left) and pyromellitic (on the right) cyclodextrin nanosponges. CDI = 1,1'-carbonyldiimidazole; CD = cyclodextrin; DMF = N,N-dimethylformamide; DMSO = dimethyl sulfoxide; Et_3N = triethylamine; NS = nanosponges; PYRO = pyromellitic dianhydride.

Figure 2. Cumulative extraction of ailanthone from the ailanthone-loaded NSs. Wt% = weight.

Figure 3. Index of growth (IGr%) of A) garden cress (*Lepidium sativum*) and B) radish (*Raphanus sativus*) in response to the application of 7.5 mg L^{-1} of pure ailanthone (Ail) (Demasi et al., 2019b) and α, γ, and LC NS-CDI loaded with Ail to provide the dose of 7.5 mg L^{-1} of Ail in the solution. Data were obtained in filter paper, at 10, 20, and 30 days after treatment (DAT). Data are means ± standard error. Similar lower-case letters within the same DAT denote no significant differences according to the Tukey post-hoc test. The statistical relevance is provided (*** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$; ns = not significant).

Figure 4. Index of growth (IGr%) of A) garden cress (*Lepidium sativum*) and B) radish (*Raphanus sativus*) in response to the application of 30 mg L^{-1} of pure ailanthone (Ail) (Demasi et al., 2019b) and α, γ, and LC NS-CDI loaded with Ail to provide the dose of 30 mg L^{-1} of Ail in the solution. Data were obtained in cultivation substrate for horticulture, at 10, 20, and 30 days after treatment (DAT). Data are means ± standard error. Similar lower-case letters within the same DAT denote no significant differences according to the Tukey post-hoc test. The statistical relevance is provided (*** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$; ns = not significant).