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Long-lasting insecticidal activity in plants driven by chlorogenic acid-loaded Metal-Organic Frameworks

Supporting information

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S1. Materials and methods

All reactants were commercially obtained and used without further purification. 2-Aminoterephthalic acid (H_2BDC-NH_2, 99%) was obtained from Acros Organics. *N*,*N*'-dimethylformamide (DMF, synthesis grade) and methanol (MeOH, extra pure) were obtained from Scharlab. Titanium(IV) isopropoxide (98%) was obtained from Thermo Scientific. Chlorogenic acid (CGA, 99.58%) was obtained from BLDpharm. Phosphoric acid (H_3PO_4, 85%) was obtained from Merck. Ethanol (EtOH, 96%) was obtained from VWR. Sodium dihydrogen phosphate (NaH_2PO4, 99%), sodium acetate (CH_3COONa, 99%), Trizma hydrochloride (Tris-HCl, 99%) and disodium hydrogen phosphate (Na_2HPO4, 99%) were obtained from Sigma-Aldrich. Acetic acid (99.8%) was obtained from Panreac. Sodium hydroxide (NaOH) was obtained from Merck.

Physicochemical characterization

Elemental analyses (EA) were carried out on a Thermo Scientific analyzer model Flash 2000. The Fourier transform infrared (FTIR) spectra, measured on powdered samples in an attenuated total reflectance (ATR) mode, were recorded on a BRUKER TENSOR 27 FTIR and OPUS data collection program. X-ray powder diffraction (XRPD) patterns of all samples were collected in an Empyream PANALYTICAL diffractometer, equipped with a PIXcel3D detector and a copper radiation source (Cu Kα, λ = 1.5406 A), operating at 45 kV and 40 mA, and in a BRUKER D8 ADVANCE equipment, where the routine PXRD conditions were from 3 to 35º (2ϑ) using a step size of 0.013º and 39525 s *per* step in continuous mode with knife and Soller slits of 0.04 rad. N_2 isotherms were obtained at 77 K using a TriStar II Plus Instruments. Before the measurement, pristine and CGA loaded MIL-125-NH_2 samples were evacuated under vacuum at 200 ºC for 16 h. Specific surface area was determined by applying Brunauer, Emmett & Teller equation (BET) in the relative pressure interval p/p_0 = 0.01–0.3 (being p_0 the saturation pressure). Thermogravimetric analyses (TGA) were carried out in an SDT Q-600 thermobalance (TA Instruments, New Castle, DE, USA) with a general heating profile from 30 to 600 ºC with a heating rate of 5 ºC min^{-1} under air using a flux of 100 mL min^{-1}. FE-SEM (Field Emission Scanning Microscope) JEOL JSM-7900F was used to obtain high resolution images with magnifications from 20-1000000x and a LED secondary electrons detector, operating at a voltage of 15.0 kv. For particle size determinations *ca.* 1 mg of material was dispersed in 10 mL of water, being analyzed with a Malvern Nano-ZS, Zetasizer Nano series. Once the suspension was prepared, a sonication step was followed (20% amplitude, 30 s), monitoring the particle size evolution during 72 h.

Synthesis of MIL-125-NH_2 or $[Ti_8O_8(OH)_4(BDC\text{-}NH_2)_6]\cdot nH_2O$.[1] 2-Aminoterephthalic acid (H_2BDC-NH_2, 1.38 g, 7.6 mmol) was dissolved in 20 mL of *N*,*N*'-dimethylformamide (DMF) and 5 mL of methanol (MeOH) at room temperature (RT) under stirring. The mixture was placed in a round bottom flask (50 mL) equipped with a condenser and was warmed at 100 °C under air. When the mixture reached the temperature of 100 °C, 1.5 mL of titanium(IV) isopropoxide (1.5 g, 5.1 mmol) was added. The mixture was kept under magnetic stirring and heated for 72 h at 100 °C under air. The yellow solid obtained was filtered and washed with DMF at RT. The as-synthesized dried product was dispersed at RT in DMF under stirring overnight (100 mL of DMF per g of product). Then, the same procedure was repeated once using MeOH instead of DMF for 4 h.

Pesticide encapsulation. Chlorogenic acid (CGA) was entrapped into de porous solids by suspending 20 mg of powdered MIL-125-NH_2 in 5 mL of a 10 mg·mL^{-1} aqueous solutions of CGA at RT under magnetic stirring for 24 h, using 30 mL vessels. The pesticide-loaded materials were recovered by centrifugation (5000 rpm for 30 min) and washed 3 times with deionized water. It should be noted that the CGA encapsulation process was optimized, and different conditions

were also tested (ethanol as solvent, or doubling soaking time (48 h)), obtaining a lower or similar CGA cargo. The amount of adsorbed pesticide was quantified by elemental analysis (EA), TGA, UV-vis spectroscopy, and high-performance liquid chromatography (HPLC). During the encapsulation process the stability of both MOFs was evaluated by quantifying the amount of released linker by HPLC. All the experiments were performed at least at triplicate. Pristine solids and CGA-encapsulated solids were characterized by XRPD, FTIR, and N_2 sorption measurements.

CGA release in aqueous media. CGA-containing materials (20 mg) were placed in 6 mL of each media (MilliQ water pH 6.5, 0.1 M sodium acetate buffer at pH 4, and 0.1 M Tris-HCl buffer at pH 8 adjusted with NaOH) at RT under magnetic stirring. At different incubation times, 3 mL of supernatant were recovered by centrifugation (40000 rpm for 5 min) and replaced with the same volume of fresh media to keep sink conditions. The, 0.2 mL of each aliquot was mixed with 0.8 mL of aqueous solution at pH 2.3, and analyzed by HPLC to determine the CGA release and the potential leached linker. This procedure was performed by triplicate. After the drug-delivery process, solid samples were characterized by XRPD and TGA.

HPLC analysis conditions. The amount of incorporated and released CGA, as well as the released linker (H_2BDC-NH_2) was determined using a reverse phase HPLC Jasco LC-4000 series system, equipped with a photodiode array (PDA) detector MD-4015 and a multisampler AS-4150 controlled by ChromNav software (Jasco Inc, Japan). For the quantification of all chemical species, isocratic conditions were used and a flow rate of 1 $mL \cdot min^{-1}$.
In the quantification of H_2BDC-NH_2, a Purple ODS reverse-phase column (5 µm x 4.6 x 150 mm, Análisis Vínicos, Spain) was employed. The column temperature was fixed at 25 ºC. The injection volume was 30 µL. The mobile phase was based on a mixture of 50 : 50 MeOH : phosphate buffered solution (PBS; 0.04 M, pH = 2.5), with a retention time (rt) of 2.7 min and an absorption maximum of 228 nm.
In the quantification of CGA, Ascentis® C18 reverse-phase column (5 µm x 4.6 x 250 mm, Supelco, Sigma-Aldrich, USA) was employed. The column temperature was fixed at 25 ºC. The injection volume was 30 µL. The mobile phase was based on a mixture 91 : 9 acetic acid(10%) : acetonitrile, with a rt and an absorption maximum of 4.4 min and 313 nm, respectively.

Preparation of the phosphate buffered solution (0.04 M, pH = 2.5). 0.02 mol (2.4 g) of NaH_2PO4 and 0.02 mol (2.84 g) of Na_2HPO4 were dissolved in 1 L of Milli-Q water. The pH was then adjusted to 2.5 with H_3PO_4 (85%) and to basic pH with NaOH (10 M).

S2. Monte Carlo calculations

In order to probe the plausible preferential adsorption sites available in the titanium terephthalate MIL-125-NH_2 structure for CGA guest molecules, force field-based Monte Carlo simulations were performed. The MOF crystal structure was taken from the literature and optimized.[2] Partial charges in the solid were then calculated using electronegativity equalization or qEq method available in Materials Studio (**Figure S1**),[3] while Lennard Jones parameters were taken from UFF to reproduce the van der Waals interactions.[4] Regarding both CGA neutral molecule and deprotonated anion (where one H from the carboxylate is removed and replaced by Na^+), Density Functional Theory (DFT) calculations were performed with DMol^3 to estimate the partial charges (**Figure S2**) obtained after a geometry optimization using PW91 functional and DNP basis set and high convergence criteria. They are combined with UFF for Lennard-Jones parameters. The CGA loading of the MOF was calculated by Grand Canonical Monte Carlo (by imposing a pressure equal to 10^4 kPa in order to assure to reach the saturation), using a temperature equal to 300 K, rigid solid and CGA structures and considering a 2x2x2 multicell (the multi cell contains thus, 128 Ti). Such a multi-cell is large enough to consider a cut-off distance for Lennard Jones interactions equal to 12.5 Å. For a typical Monte Carlo calculation, $5 \cdot 10^6$ steps for both, equilibration and production processes, were used. From these calculations, it was therefore possible to extract the configuration of CGA distribution in the MOF pores and determine the plausible interactions.

In the empty and saturated structures, it was also possible to calculate the textural properties (specific surface area for N_2, free pore volume), using the procedures developed by Düren *et al*.[5] (considering the center of a N_2 probe molecule rolling across the surface with a diameter equal to 3.681 Å) and Gubbins *et al*. (probe size of 0 Å),[6] respectively.

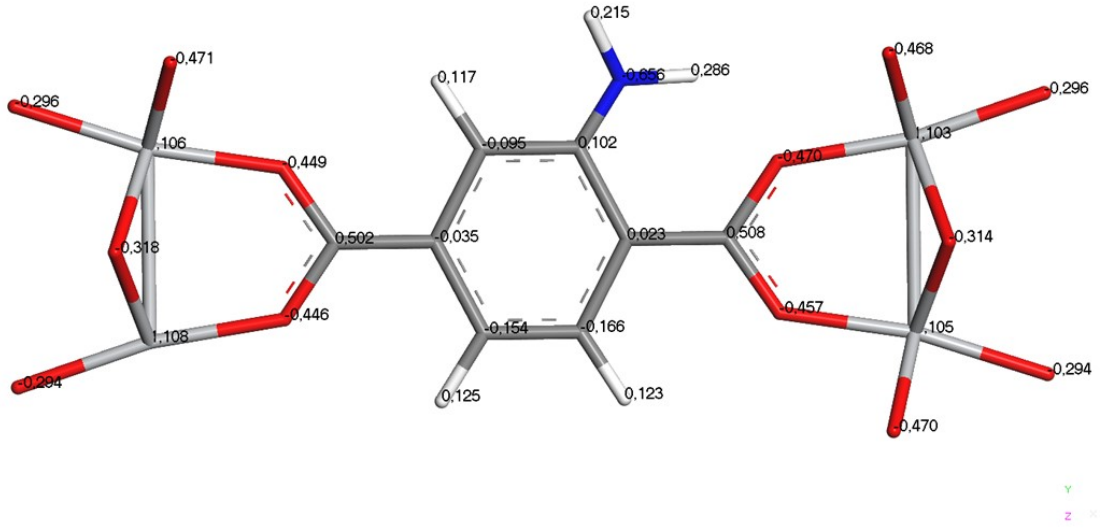

Figure S1. Partial charges obtained from qEq calculations for MIL-125-NH_2.

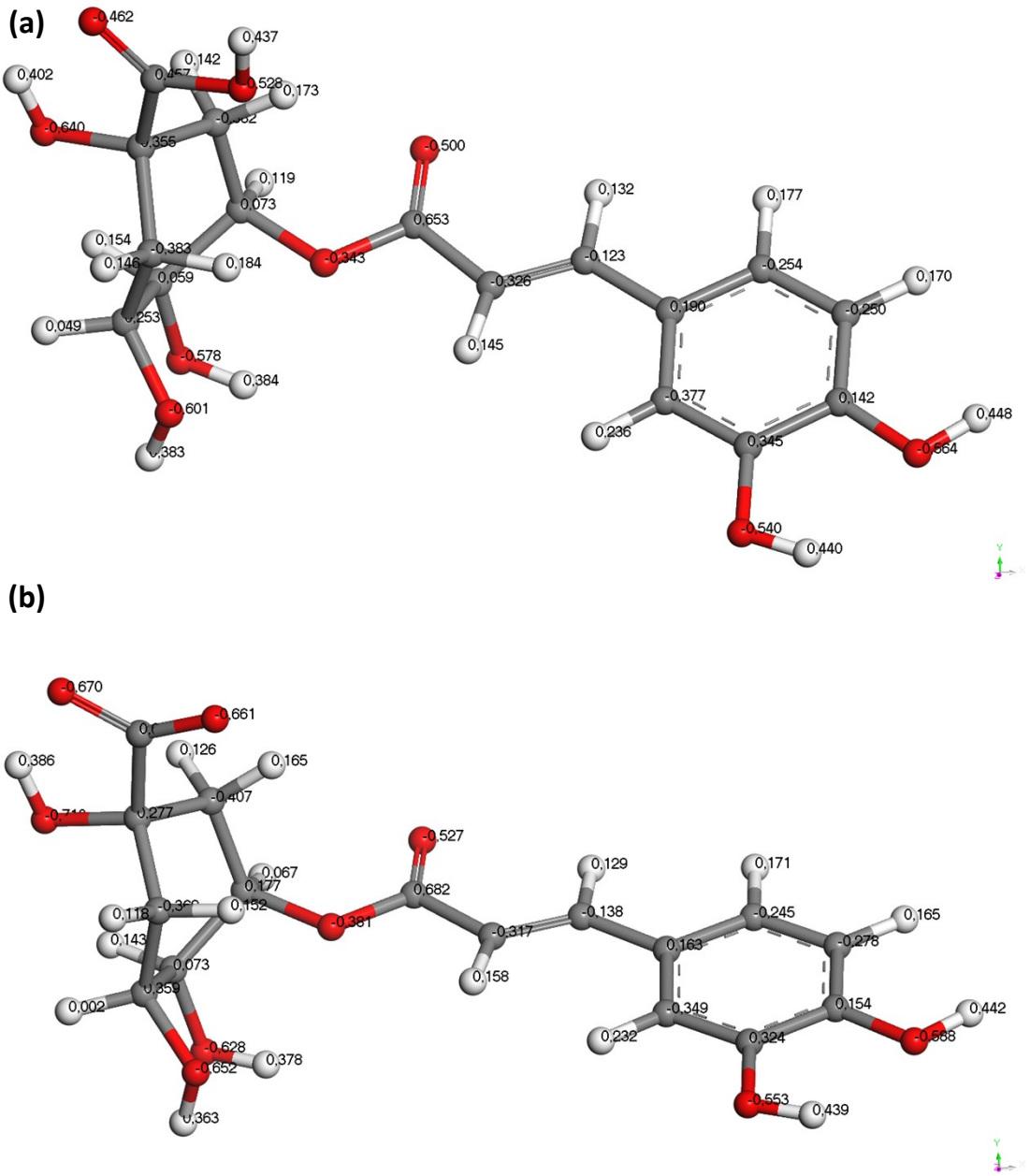

Figure S2. Partial charges obtained from DFT calculations for (a) neutral CGA**,** and (b) ionic CGA.

S3. HPLC conditions

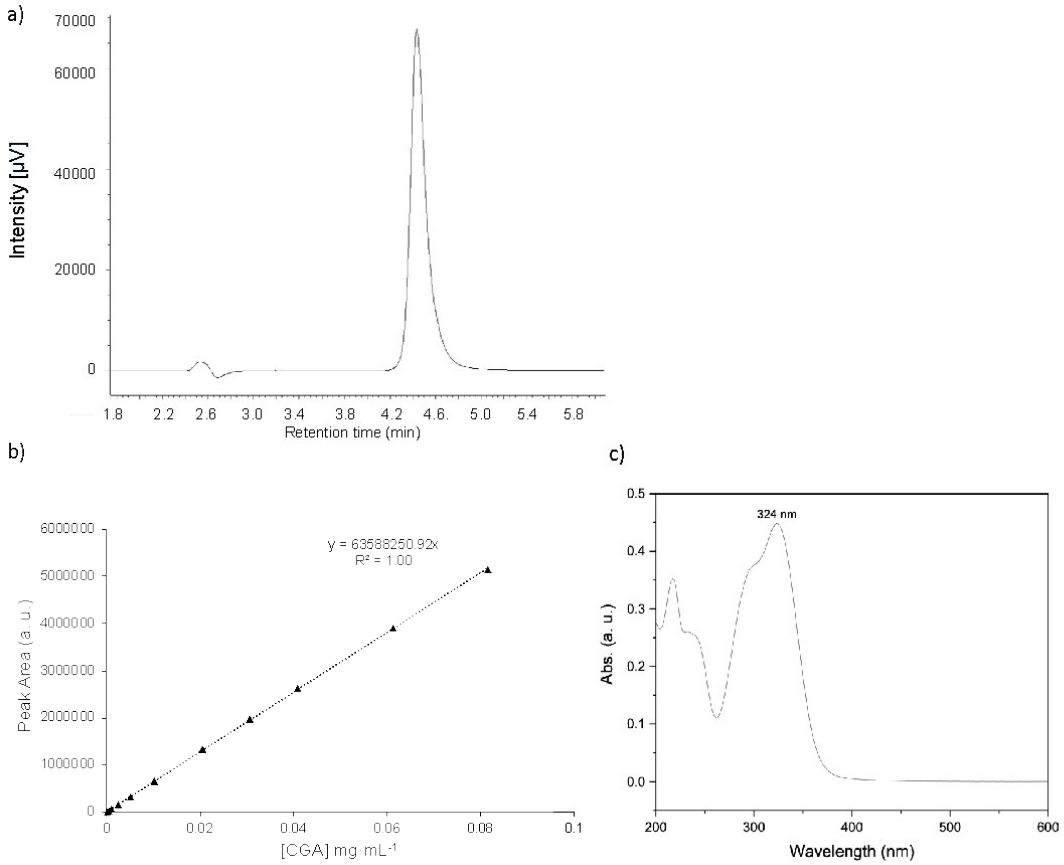

Figure S3. (a) HPLC chromatogram, (b) calibration plot of standard by HPLC method, and (c) UV-vis spectrum of CGA.

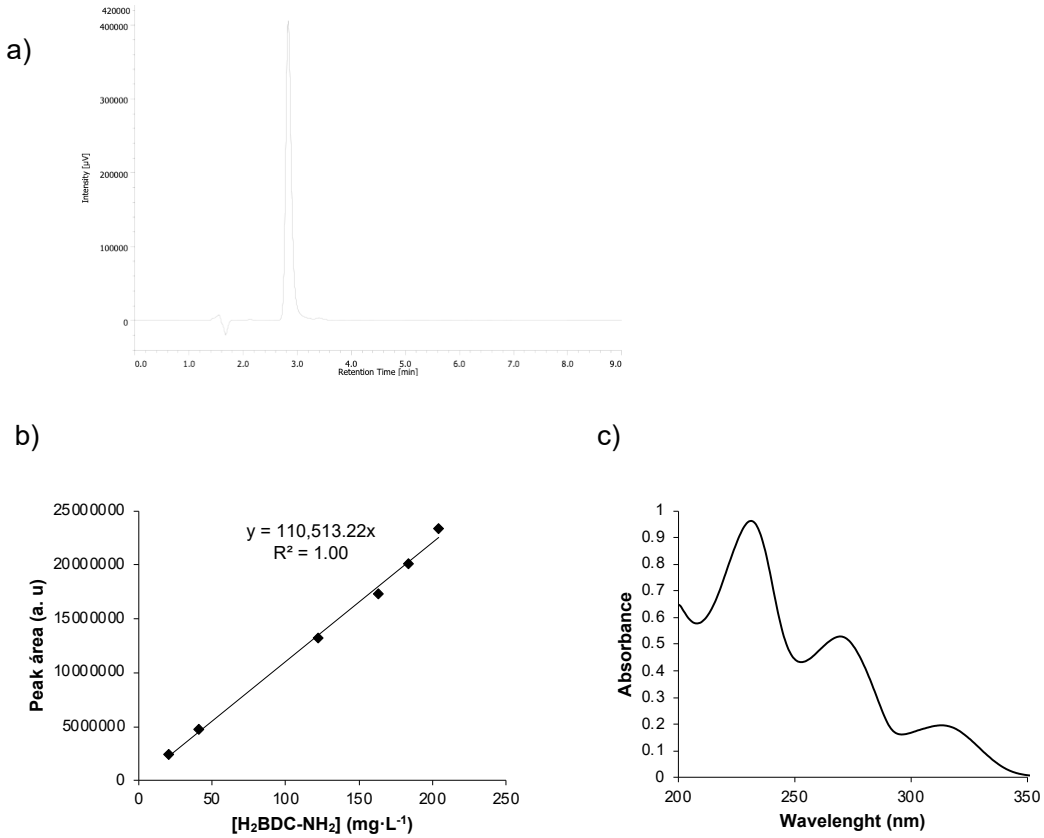

Figure S4. (a) HPLC chromatogram, (b) calibration plot of standard by HPLC method, and (c) UV-vis spectrum of $H_2BDC\text{-}NH_2$."

S4. Materials characterization

Elemental analysis

Elemental analysis (C, H and N) for CGA@MIL-125-NH_2: Theoretical (%): C 36.76; H 3.71; N 3.28. Experimental (%) C 36.12; H 4.95; N 3.45. Proposed formula for CGA@MIL-125-NH_2: $[Ti_8O_8(OH)_4(O_2CC_6H_3NH_2CO_2)_6](C_{16}H_{18}O_9)_{1.9}(H_2O)_{13}$. MW: 2561.12 g·mol^{-1}.

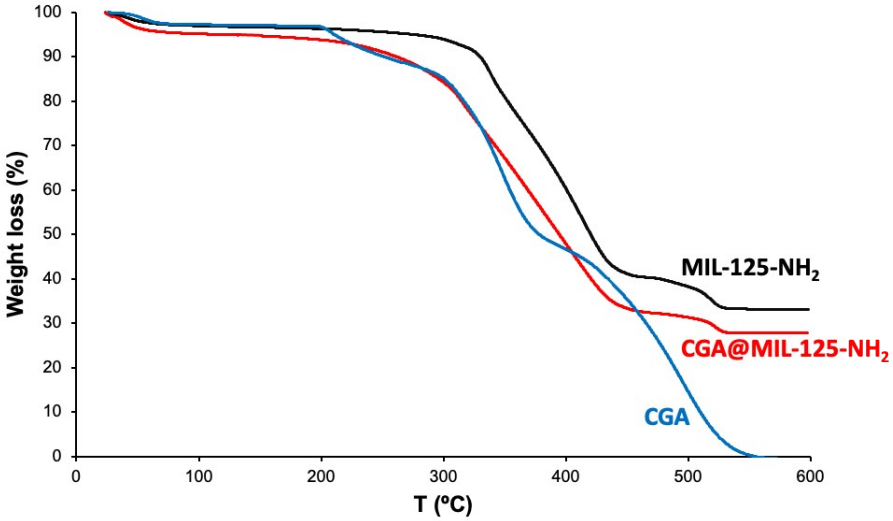

Figure S5. TGA of MIL-125-NH_2 (pristine material in black), as well as their corresponding CGA loaded material (red), and the free CGA (blue).

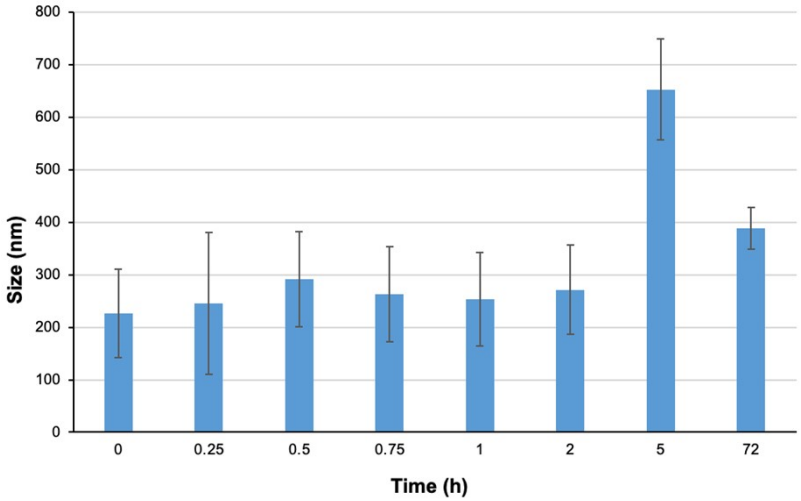

Figure S6. Particle size (with standard deviation) of the CGA@MIL-125-NH_2 in water.

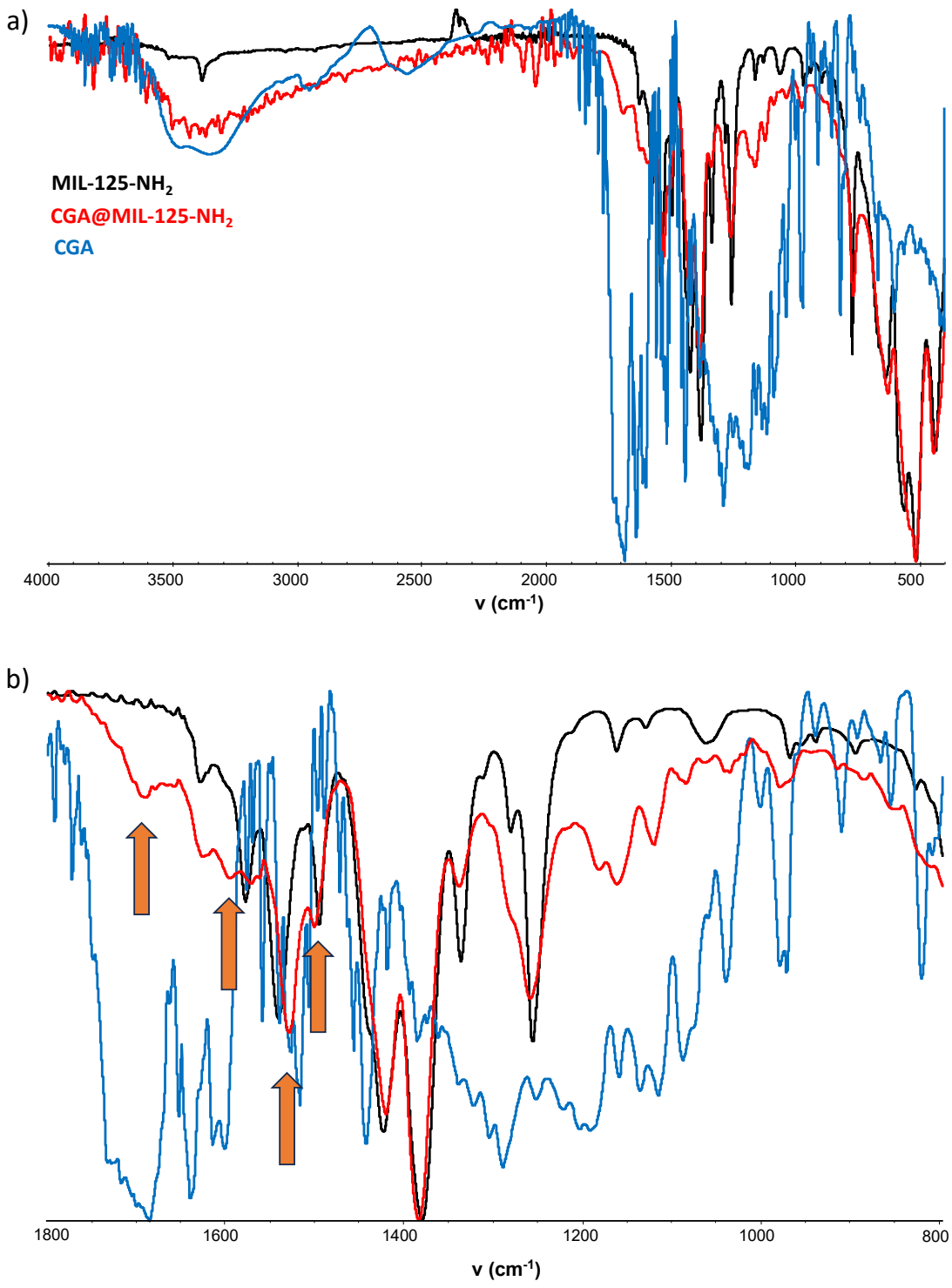

Figure S7. FTIR spectra of MIL-125-NH_2 before (black) and after (red) CGA incorporation compared with free CGA (blue), a) from 4000 to 400 cm^{-1}, and b) from 1800 to 80 cm^{-1}.

S5. CGA delivery

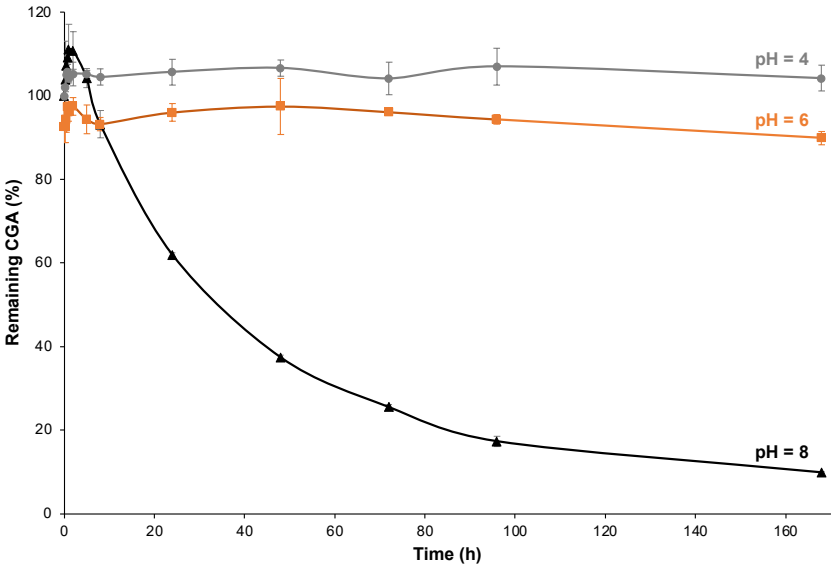

Figure S8. Remaining CGA when solubilized in aqueous media at different pH values for 7 days: pH = 4 (grey, circles), pH = 6 (orange, squares), and pH = 8 (black, triangles).

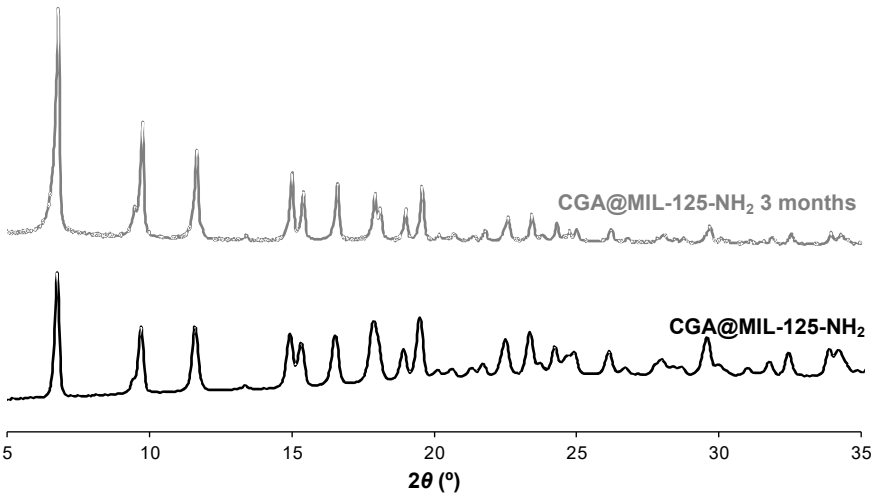

Figure S9. XRPD patters of freshly prepared CGA@MIL-125-NH_2, and after 3 months of storage.

The Higuchi model, which defines the short time behaviour of the release of a dispersed cargo from a homogenous matrix has been used to describe the diffusion of the CGA from the MOF matrix.[7] Considering that the external surface diffusion process around the MOF particles is minimized by continuous stirring during the delivery assay, the desorption process might only be due to the drug movements through the pores of the framework. The CGA release from CGA@MIL-125-NH_2 could be explained by the following equation:

$$[CGA] = K \cdot t^{1/2}$$

where [CGA] corresponds to the concentration of released CGA (mg·g^{-1}), *t* is the time (h), and *K* is the kinetic constant (g·mg^{-1}·$h^{-1/2}$). The CGA release can be empirically adjusted in the first hour to the Higuchi model with regression factor (R^2) > 0.99.

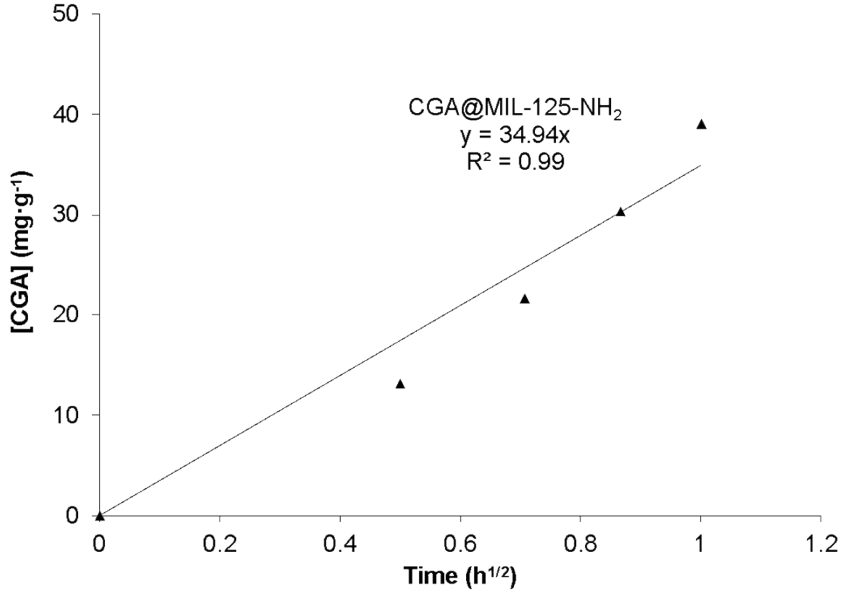

Figure S10. Fitting of the CGA delivery data from CGA@MIL-125-NH_2 to the Higuchi model.

S6. Effect of MIL-125-NH_2 on seed growth

The potential growth effect of MIL-125-NH_2 was tested with *Lolium multiflorum* seeds (annual ryegrass, Batlle, used here as model plant). Aqueous suspensions (20 mL) of MIL-125-NH_2 with different concentrations (50, 250, 500 and 750 ppm) were tested. In parallel a water control was performed. Seeds were kept at room temperature for 7 days. 7 cm^2 Petri dishes were used for each concentration with a total of 10 seeds per petri dish. The length of the stem and roots of plants were measured using ImageJ software. Then each group of plants was dried for 24 h at 100 °C to see differences in dried plants weight. The statistical analysis was performed using the one-way ANOVA with a p-value<0.05.

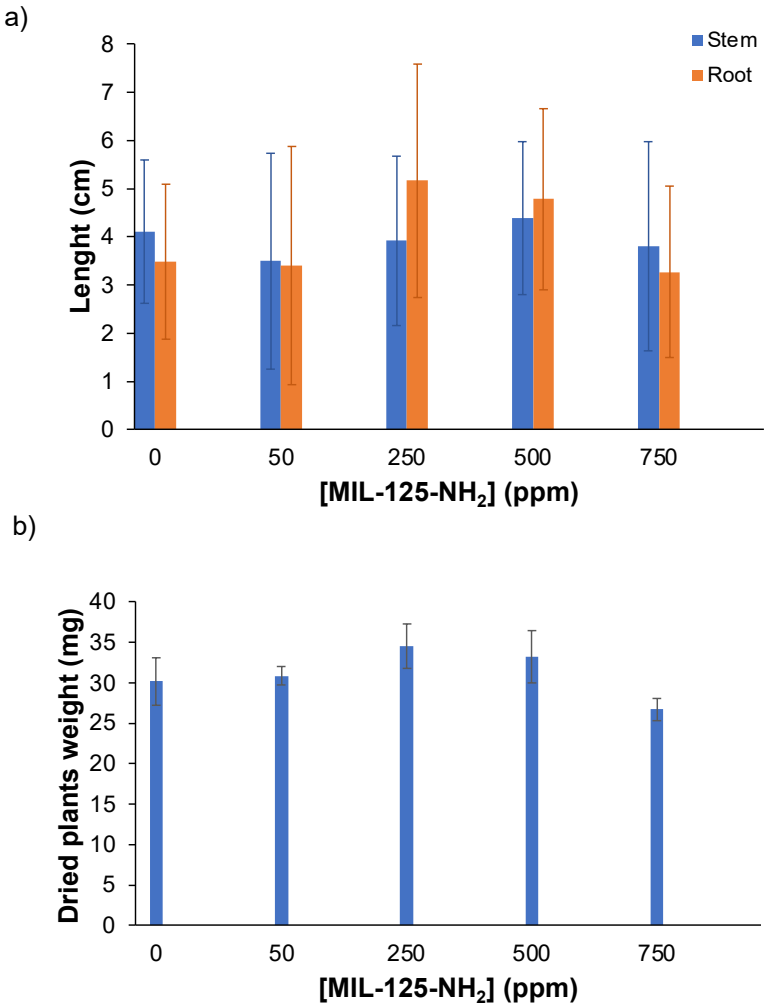

Figure S11. (a) Effect of MIL-125-NH_2 in plants stem and root length (cm), and (b) dried plants weight (mg).

S7. Insecticidal biological assay

The potential insecticidal activity of CGA@MIL-125-NH_2 was tested against mealworm (*Tenebrio molitor*), which are often used as laboratory insect test for bioassays.[8,9] Mature larvae of *T. molitor* were obtained from a local insect breeding distributor, maintained in plastic boxes at air-conditioned room (22-25 ºC), under dark and fed with oat.

Previous to any test, the active concentration of CGA against *T. molitor* was determined considering previous effective CGA concentrations against herbivores found in leaves of *Salix pentandra* or *Solanum licopersicum*.[10,11] Aqueous solutions (1 mL) of CGA with different concentrations were tested. Different glasses with parafilm on the bottom were used for each different concentration with a total of 20 larvae *per* glass. *T. molitor* mortality was studied for 21 days. It should be noted that CGA is a naturally occurring antioxidant dietary polyphenolic compound normally ingested as it is found at high concentration in plants, fruits, vegetables or coffee, and therefore, the selected concentration is completely safe (reported oral median lethal dose LD_{50} in rats >2000 mg·Kg^{-1}).[12]

Once the active concentration of CGA was determined, four different groups of 20 larvae denoted as oat (negative control), CGA (positive control), MIL-125-NH_2, and CGA@MIL-125-NH_2 were studied. For each group, the administered amount of material was adjusted to the corresponding part of CGA (*e.g.,* 50 mg of CGA *per* g of oat corresponds to 170.8 mg of CGA@MIL-125-NH_2 with a 29.27%wt. of CGA). Larvae were fed with repeated doses of oat previously doped with 2 mL of the corresponding suspension. In summary, the studied groups were:

Oat (negative control): 20 larvae were fed with repeated doses (0, 1, 2 and 5 days) of oat (1 g).
CGA (positive control): 20 larvae were fed with repeated doses (0, 1, 2 and 5 days) of oat (1 g) previously doped with 2 mL of an aqueous suspension of CGA (total 200 mg).
MIL-125-NH_2: 20 larvae were fed with repeated doses (0, 1, 2 and 5 days) of oat (1 g) previously doped with 2 mL of an aqueous suspension of MIL-125-NH_2 (total 441.2 mg).
CGA@MIL-125-NH_2: 20 larvae were fed with repeated doses (0, 1, 2 and 5 days) of oat (1 g) previously doped with 2 mL of an aqueous suspension of CGA@MIL-125-NH_2 (total 683.2 mg).

For calculations and statistical analyses, lethal time (LT) was determined using the Probit analysis.[13] The lethal times LT_{50} meaning time to kill 50% individuals were calculated. These statistical analyses were performed with IBM SPSS Statistics (version 28.0.1.0).

Table S1. Effect of the application of oat, and oat supplemented with CGA, MIL-125-NH_2 and CGA@MIL-125-NH_2 on larvae of *T. molitor* in a 21-day laboratory assay.

Group	Median lethal time, LT_{50} (days)
Oat	52.2
CGA	7.5
MIL-125	17.3
CGA@MIL-125-NH_2	16.1

Treated and control larvae groups were placed in glasses with parafilm on the bottom, incubated in a climatic chamber at (22-25 ºC). Mortality was recorded before the addition of each dose of oat for 21 days. All biological assays were repeated 3 times. The significance of data distribution was tested by one-way ANOVA test. Data are shown as the mean and the standard deviation. A value of $p < 0.05$ was considered statistically significant.

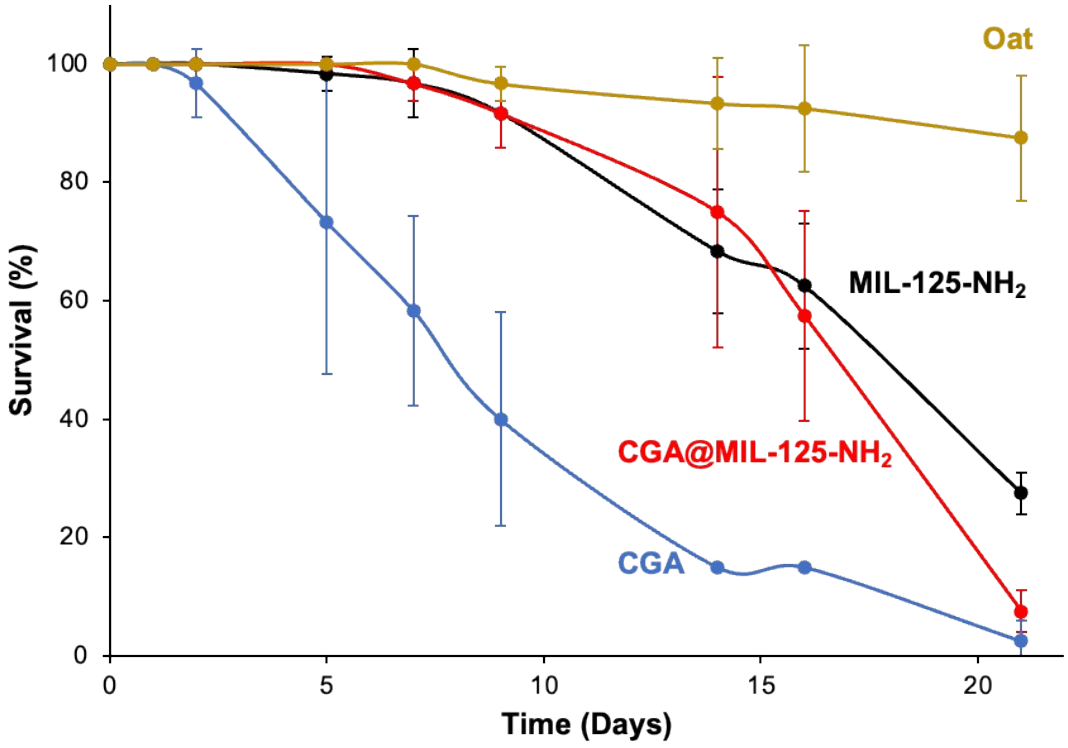

Figure S12. Survival (%) of *T. molitor* larvae after 21 days of the first administration of different materials: Negative control (oat), positive control (free CGA), MIL-125-NH_2, and CGA@MIL-125-NH_2.

S8. References

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