Supplementary Information (SI) for Environmental Science: Nano.

Supporting information (8 pages, 10 Figures) for

A novel engineered nanoherbicide: improving performance, efficiency and sustainability of herbicide Bentazon

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Calibration Curves

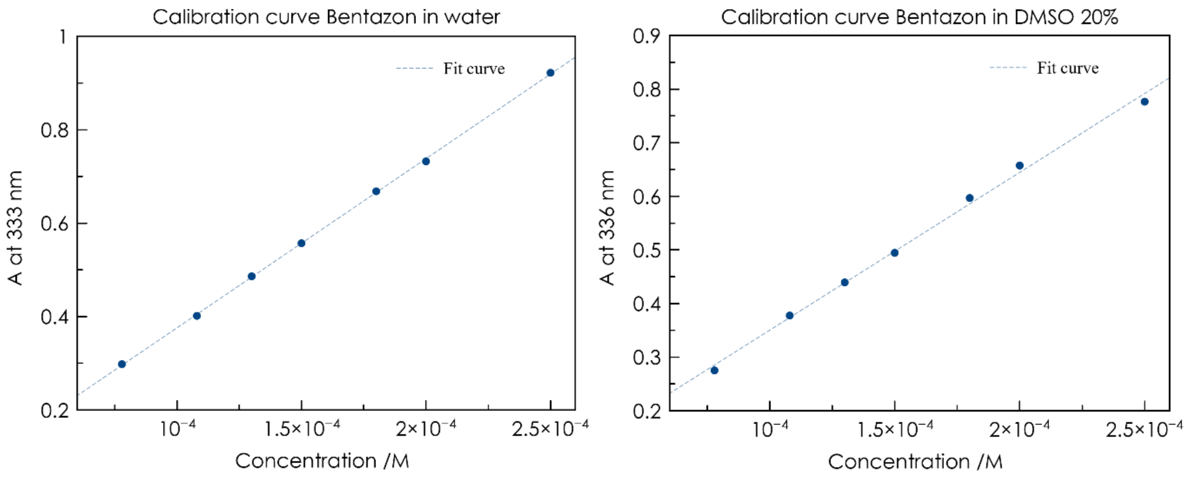


Figure SI-1. Calibration curve of Bentazon in water and DMSO/water 20%. The fitting linearization resulted in y = 3628,2x + 0,0134 R^2 = 0,9998 and y = 2948,7x + 0,0556 R^2 = 0,9963 respectively. The second calibration curve was used to assess indirectly the bentazon charge load from the supernatant from adsorption procedures. The results showed minimal discrepancy with the Elemental Analysis that were thus selected as most accurate method.

Analytical methods validation

The following are the quality assurance (QA) and quality control (QC) operations performed to validate the analytical instruments (Elemental analyser and Agilent Cary 60 UV-Vis Spectrophotometer) and establish the methods for samples quantitative analysis (QA/QC):

CHNS Elemental Analyzer: The amount of bentazon in Btz-ACP was calculated by analysing its nitrogen content by CHNS elemental analysis with a Thermo ScientificTM Flash 2000 CHNS Analyzer from CIC-UGR of University of Granada. The equipment maintenance (replacement of copper or chromatography column) is periodically conducted by qualified technician previously trained by Thermo Scientific staff. The linearity, precision and accuracy is periodically verified by trained technician by measuring three replicates of three standards of sulphanilamide and a blank (empty tin capsule). Then, to validate the calibration method, a sample of sulphanilamide of known concentration is analysed.

UV-Vis spectroscopy: Agilent Cary 60 UV-Vis Spectrophotometer was used to monitor bentazon release in different media. QA/QC test is carried out periodically according to the instructions of the manufacturer (Agilent). The wavelength accuracy is tested by measuring periodically three repeated wavelength scans of holmium oxide solution in perchloric acid (from 240 to 650 nm). The peak positions are cross-checked with the certified data for this standard, with a wavelength accuracy of ± 0,5 nm. The wavelength precision refers to the standard deviation of three replicates of the absorbance peaks, being better than 0,2 nm across the operational range of the instrument.

The absorbance accuracy, precision and linearity are confirmed for the intended operational range by measuring three replicates of standard potassium dichromate solutions at three different concentrations 40, 80 and 120 mg/L. The absorbance accuracy must be within the mean ± 0,002 Abs (Abs=0-0,500) and ±

0,004 Abs (Abs>0,500). The linearity is confirmed with a correlation coefficient greater or equal to 0,999.

PXRD diffractograms

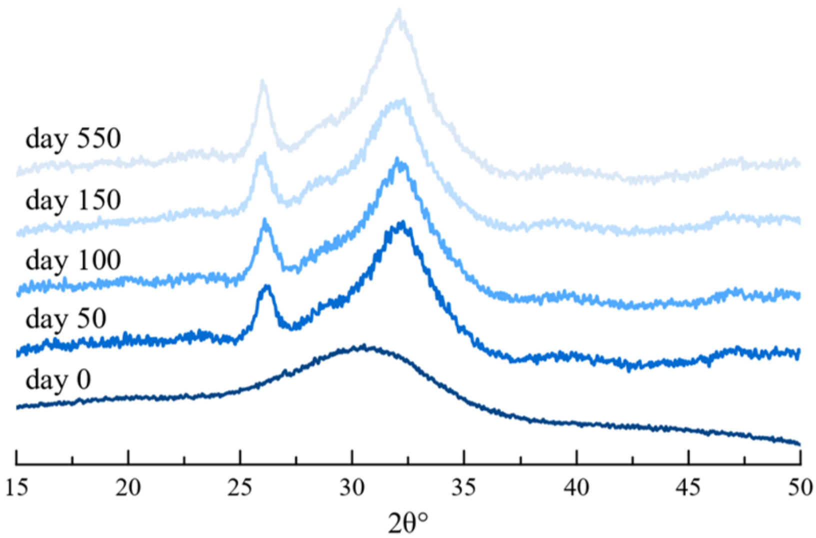


Figure SI-2. Powder diffractograms of ACP before functionalization (green) and Btz-ACP at different maturation times (blue shades). The samples were kept as synthesized in wet conditions (i.e., 10 % *w/w* nanoparticles and 90 % *w/w* water) at 4 ºC. As it can be noticed the amorphous form is eventually evolving in the poorly crystalline apatite in the timeframe of 50 days. However, no recrystallization of Btz is observed even at much longer times.

TEM images

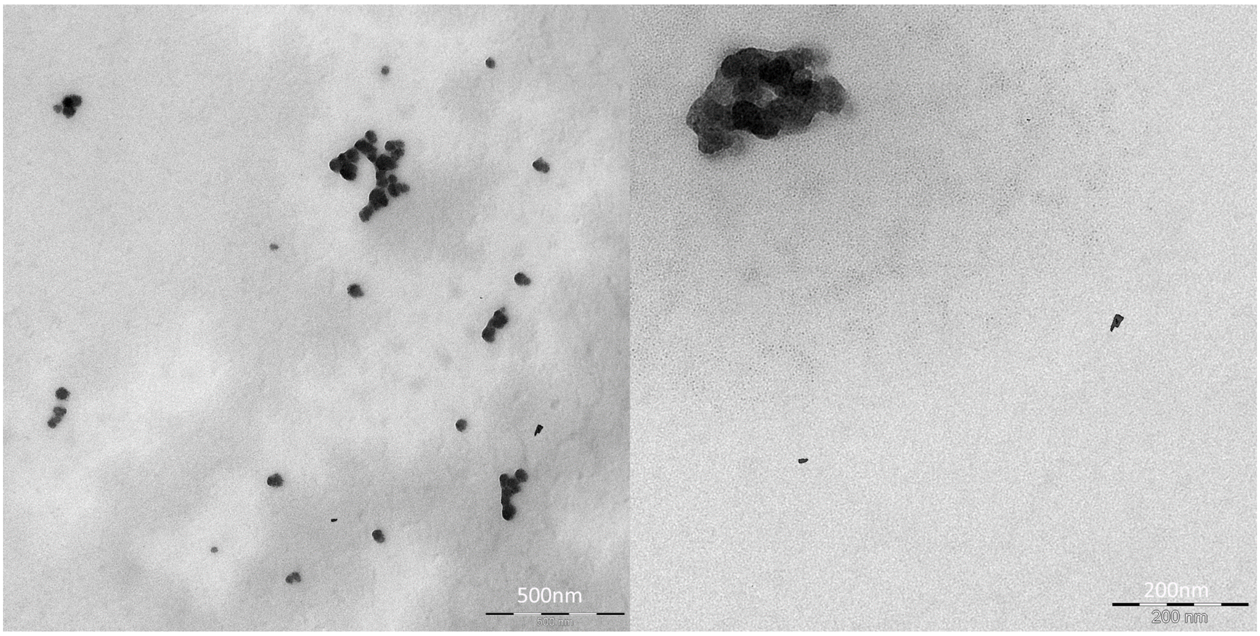


Figure SI-3. TEM micrographs of **ACP**.

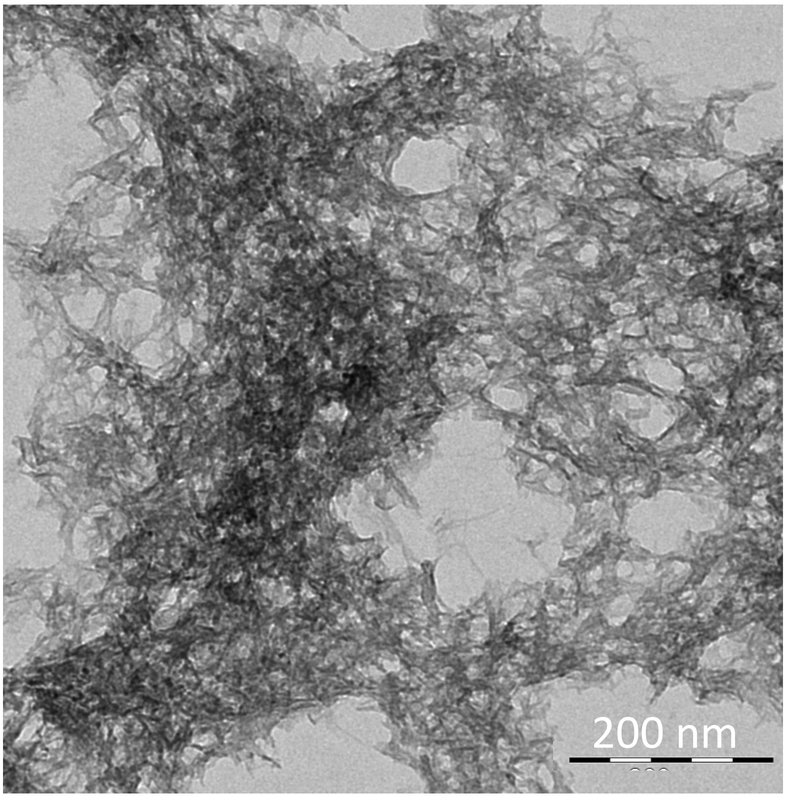


Figure SI-4. TEM micrograph of **Btz-ACP**.

pH dependent release in aqueous media

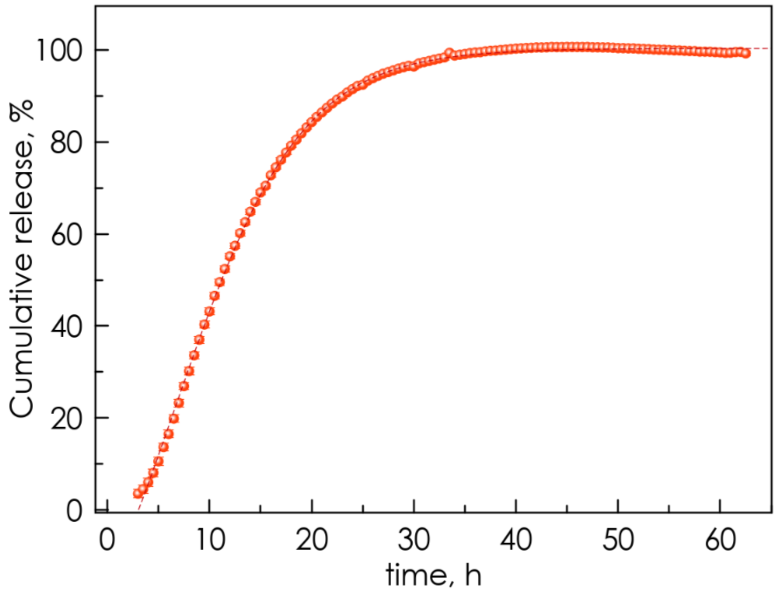
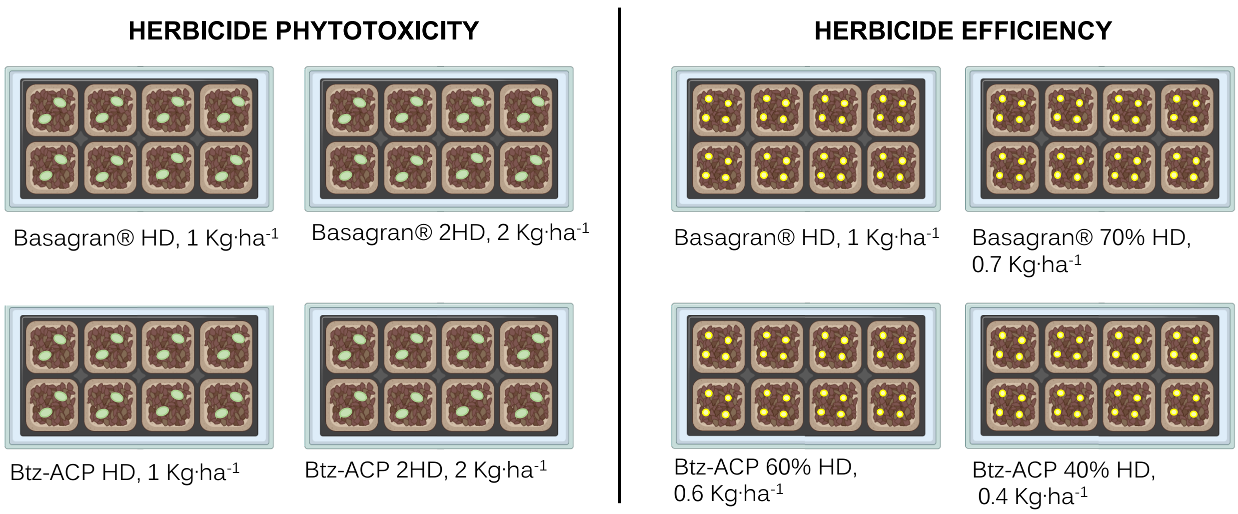


Figure SI-5. Btz release in aqueous media at pH of 4,5 in acetate buffered solution, experimental results are average of triplicates and errors are reported in the graph. Dotted line represents the best fit to the experimental data. Release constant resulted in k=0,20 h^{-1} for the release in acetate buffer. This result show a 2 times higher kinetics than in neutral Tris-HCl buffered media.

In vivo experiments

Design of the experiments



Scheme SI-1. The higher dose (HD) of Basagran® 87% is 1,15 Kg·ha-1 that corresponds to 1 Kg·ha-1 of bentazon and 1,13 mg/pot of bentazon. Phytotoxicity experiments were applied on crop plants (Pisum sativum) and efficiency tests on weeds (*Sinapis alba*). For each experiment, a control with the higher dose was applied, water in the case of commercial bentazon experiments and non functionalised nanoparticles in the case of nanoherbicide treatment.

Table 1. Summary of applied doses of herbicide with reference to active ingredient Btz quantity.

Suggested higher dose Basagran®	1,15 kg/ha	
Treatment	kg Btz/ha	mg Btz/pot
HD	1,00	1,13
70% HD	0,70	0,79
60% HD	0,60	0,68
40% HD	0,40	0,45
2 HD	2,00	2,26

Phytotoxicity experiments

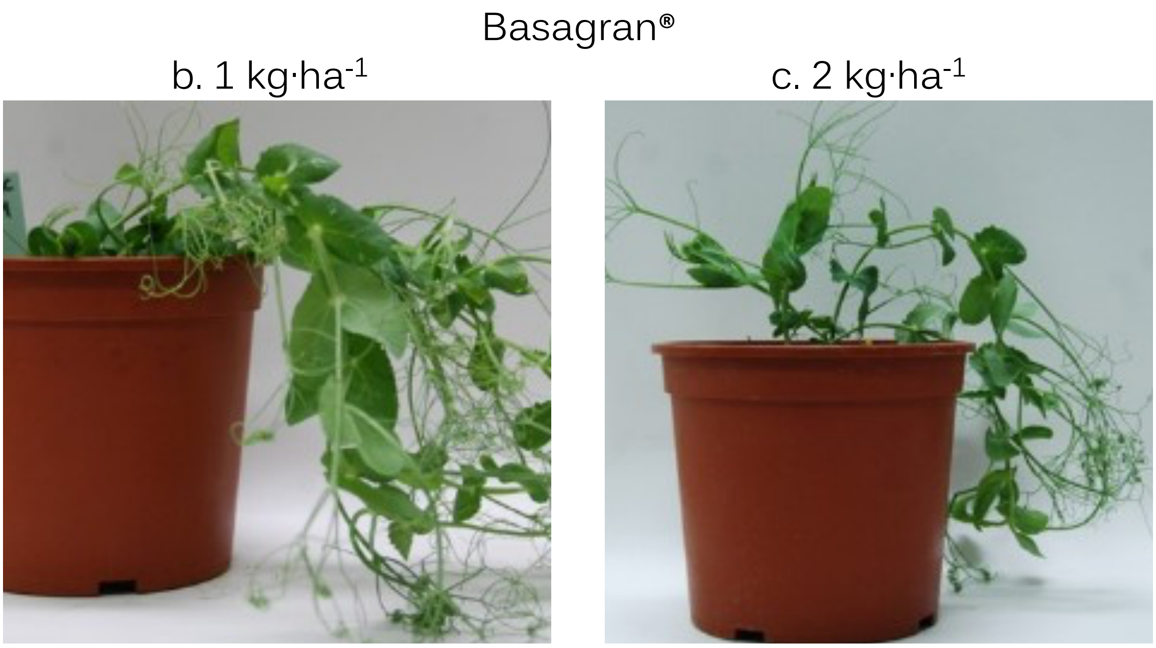


Figure SI-6. Representative crop *Pisum sativum* L. plants of phytotoxicity treatments with Basagran HD (1 kg·ha^{-1}) and 2HD (2 kg·ha^{-1}).

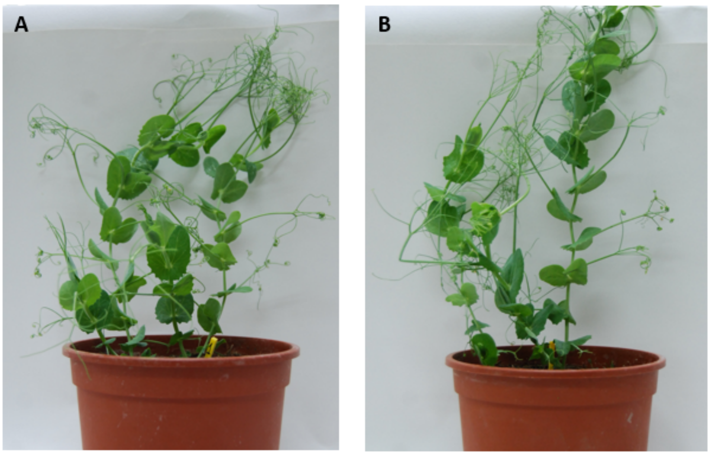


Figure SI-7. Control *Pisum sativum* plants for phytotoxicity experiments treated with water (A) and naked ACP nanoparticles resuspended in water (B) in the legume crop pea.

Efficiency tests

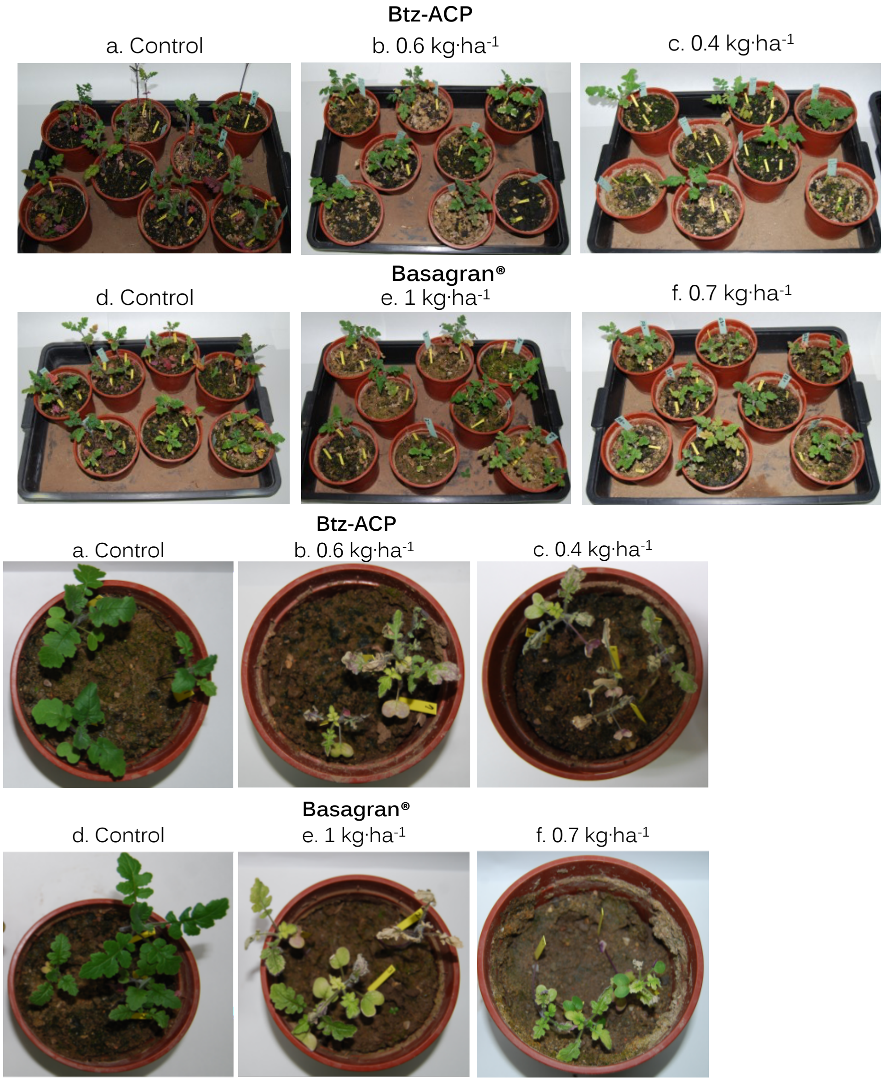


Figure SI-8. General view and zoomed view of the white mustard plants 28 days after treatments.

Variety of symptoms

Figure SI-9. a) Variety of symptoms on *Sinapis alba* after herbicide application, either as Btz or as Btz-ACP such as necrosis spots, larger necrotic areas, and chlorotic areas of varying extent; b) new sprouts 28 days post treatment.

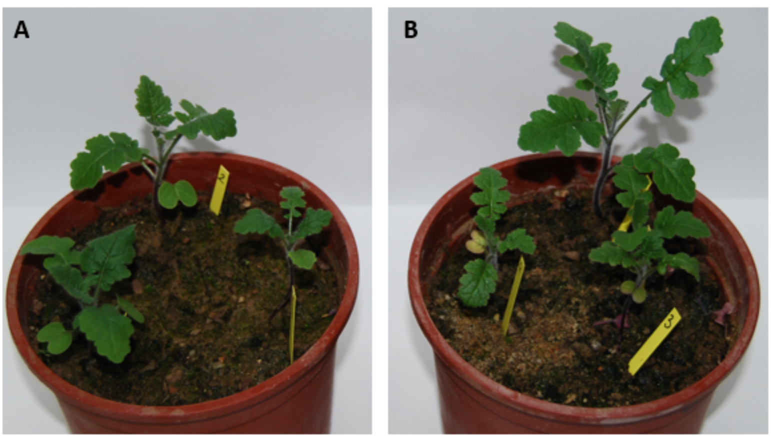


Figure SI-10. Control plants of *Sinapis alba* treated with water (A) and empty ACP nanoparticles resuspended in water (B) in the white mustard weed.