Supplementary Information

A Multi-Stimuli-Response Metal-Organic Framework Nanopesticide for Smart Weed Control in Agriculture

Dingyang Zhang[1], Xueping Guo[1], Wenhua Rao[3], Danmei Pan[4], Fang Cao[1], Tianyun Zhai[1], Wenhui Zheng[1], Yakubu Saddeeq Abubakar[1, 5], Xiong Guan [1], Zhi Chen[2,*], and Xiaohong Pan[1,*]

[1] *State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops College of Plant Protection & Key Lab of Biopesticide and Chemical Biology, Ministry of Education, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, P. R. China*

[2] *College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, P. R. China*

[3] *Institute of Plant protection, Fujian Academy of Agriculture Sciences, Fuzhou 350003, P. R. China.*

[4] *Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou, Fujian 350002, P. R. China*

[5] *Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria 810281, Nigeria.*

*Corresponding Authors

E-mail: panxiaohong@163.com (Xiaohong Pan);

Email: chenzhi0529@163.com (Zhi Chen);

SUPPLEMENTAL METHODS

The ultraviolet scanning spectra of ZIF-8, BIS, and BIS@ZIF-8

The UV-vis absorption spectrum of BIS was determined through a systematic procedure. Initially, a precise amount of BIS was measured using an electronic balance. Subsequently, the BIS was dissolved in distilled water at room temperature, with the distilled water serving as a blank background for zero adjustment. The UV spectrum was then scanned in the wavelength range of 190 to 500 nm using a UV-vis spectrophotometer. Following the scanning process, an absorption curve for BIS was plotted, allowing for the identification of the maximum absorption wavelength.

Additionally, the same procedure was employed to determine the maximum absorption wavelengths of ZIF-8 and the BIS@ZIF-8 complex. This involved measuring an appropriate quantity of each substance, dissolving them in distilled water, and scanning their UV spectra within the specified wavelength range. The absorption curves for ZIF-8 and BIS@ZIF-8 were then plotted, aiding in the identification of their respective maximum absorption wavelengths. The comparison of these spectra provided valuable insights into the absorption characteristics of BIS, ZIF-8, and the BIS@ZIF-8 complex.

The BIS standard curve preparation

103.9 mg of BIS (96.2%) was accurately weighed with an electronic balance, dissolved in 100 mL distilled water and PBS at pH 7.4 and 5.0, respectively, to prepare 1000 μg/mL BIS mother solution, and then diluted step by step into a series of solutions of 25, 20, 15, 10, 5, 2.5 and 1 μg/mL. The absorbance of BIS solutions with different

concentrations was measured at the maximum absorption wavelength. Using BIS concentration as abscissa and UV absorbance value as ordinate, the standard curves of BIS in aqueous solution, pH 7.4, and 5.0 PBS solution were obtained by the linear regression equation. The standard curve for BIS using HPLC and the instrument conditions were as follows: HPLC System: Waters e2695-2489 liquid chromatograph (Waters Corporation); Column: DiMa C18 (5 μm, 150 × 4.6 mm); Mobile Phase: Acetonitrile/phosphoric acid solution (0.05%) = 55:45 (v/v); Flow Rate: 1 mL/min; Detection Wavelength (UV): 247 nm. Subsequently, we selected three BIS samples and performed both HPLC and UV-Vis tests on them, calculating the BIS concentrations obtained from each method.

Synthesis of FITC-Labelled ZIF-8 and FITC-Labelled BIS@ZIF-8

FITC-labelled ZIF-8 (FITC@ZIF-8) was prepared following the reported procedure with minor modifications[1]. Briefly, 1 mL of ethanolic 0.1% (w/v) FITC and 8 g of 2-methylimidazole were dissolved in 32 mL of deionized water under stirring at room temperature. Afterward, 3.2 mL of $Zn(NO_3)_2 \cdot 6H_2O$ (250 mg/mL) solution was dropwise added to the mixture and stirred at 1200 r/min for 2 h (25°C). The FITC@ZIF-8 nanoparticles were harvested by centrifugation (10000 rpm for 10 min), and washed with deionized water until the supernatant had no fluorescence. FITC-Labelled-BIS@ZIF-8 was performed according to the following experimental procedure[2, 3]. Briefly, 1 mL of ethanolic 0.1% (w/v) FITC and 20 mg BIS@ZIF-8 were dissolved in 20 mL of deionized water. After stirring for 1 h, FITC-labeled-BIS@ZIF-8 nanoparticles were centrifuged at 10000 rpm for 10 min and washed by deionized water

to remove fluorescence in the supernatant. It is worth noting that the FITC-labeled-nanoparticles were stored at room temperature and covered with aluminum foil to protect FITC from bleaching.

Weeding effect after simulated rainwater scouring

5 mL BIS (0.1 mg/mL) and BIS@ZIF-8 (1.0 mg/mL) aqueous solutions were uniformly sprayed on the leaves of barnyard grass. Then 5 mL PBS (pH 5.0) was uniformly sprayed to simulate rain erosion. The same size leaves that had not been rinsed with PBS were used as controls. The barnyard grass was incubated in an artificial climate incubator (T=26±3°C, RH=60±5%, L: D=16: 8), the weeding effect was observed and the plant height, fresh weight and chlorophyll content of barnyard grass were determined after 10 days of culture, and the chlorophyll content was evaluated by a portable chlorophyll meter (TYS-A, Beijing Zhongkeweihe Technology Development Co., Ltd.).

Weeding effect after UV irradiation

5 mL BIS (0.1 mg / mL) and BIS @ ZIF-8 (1.0 mg / mL) aqueous solution were evenly sprayed on grass leaves. The weeds were dried naturally after treatment, and then exposed to simulated ultraviolet light for 4 h, respectively, and then cultured in an artificial climate incubator (T = 26 ± 3 °C, RH = 60 ± 5 %, L : D = 16: 8) to observe the weeding effect. After 10 days, the plant height, fresh weight and chlorophyll content of barnyard grass were measured. The treatment with complete darkness for 4 h was used as a control. All experiments were repeated for three times.

UV degradation of ZIF-8

The experiment of ZIF-8 under UV irradiation referred to the method previously reported.[4, 5] The water stability of ZIF-8 nanoparticles in deionized water (18 MΩ) with a powder-water mass ratio of 0.75 wt % at room temperature under simulated ultraviolet light irradiation and without irradiation was studied. The ultraviolet light of the irradiation device is composed of UVA lamp (wavelength 352 nm) and UVB lamp (wavelength 306 nm). The intensity is 15 W, and the lamp is 20 cm away from the irradiation platform. In order to ensure the uniformity of irradiation, the irradiation platform can be continuously rotated, and an exhaust fan is installed to ensure the constant temperature in the device. The hexahedron (3 cm×3 cm×3 cm) containing ZIF-8 aqueous suspension was placed in an ultraviolet irradiation device. As a control sample without light irradiation, a bottle of ZIF-8 aqueous suspension was completely covered with aluminum foil and placed in an ultraviolet irradiation device. Then, 1 mL suspension was taken out at the corresponding time (1, 2, 3, 4, 5, 6, and 12 h) and the nanoparticle suspension (0.2 μm membrane) was filtered by syringe. The concentration of 2-methyl-imidazole (HmIm) released from the filtrate was detected by UV-Vis spectrophotometer, and the degradation rate of ZIF-8 was calculated.

Biosafety evaluation of BIS@ZIF-8

To recognize the effects of BIS@ZIF-8 on the growth and development of rice, 5 mL of BIS@ZIF-8 aqueous suspension (1.0 mg/mL), BIS aqueous solution (0.1 mg/L), ZIF-8 aqueous solution, and deionized water were sprayed onto the rice leaves at room temperatures, respectively. The treated rice was cultured in an artificial climate incubator (T=28±2°C, RH=70±5%, L: D=16: 8) for 10 days, and the plant height, fresh

weight, and chlorophyll content of rice were measured. To determine the activity of rice enzymes after different treatments, 0.1 g of rice leaf tissue was homogenized in 50 mM phosphate buffer with pH 7.0 prior to centrifugation at 9500 rpm for 15 min. Superoxide dismutase (SOD) activity was assessed at 560 nm,[6] peroxidase (POD) activity was observed at 470 nm, and the activity of catalase (CAT) was measured at 240 nm through a UV-vis spectrophotometer.[7] The malondialdehyde (MDA) content was determined at 532 nm.[8] All tests were carried out at least in triplicate.

The biosafety of BIS@ZIF-8 on non-target pest was also evaluated. ZIF-8 suspensions (2 mL) with different concentrations (0.01, 0.06, and 0.11 mg/mL) were evenly sprayed on the same size of mulberry leaves, and the clean water was served as the control treatment. After natural drying, one second-instar silkworm was added to each well (n=10/treatment). Finally, after incubation in the environmentally controlled room (T=26±1°C, RH=60±5%, L: D=16: 8) for 15 days, the body length of the silkworm was measured by a digital caliper. All the treatments were carried out in triplicate.

The goldfish was selected as aquatic organisms to test the biosafety of BIS@ZIF-8. Healthy goldfish with consistent growth (n=6) were selected and distributed into a 5 L basin with different concentrations of ZIF-8 aqueous solution (0.01, 0.06, and 0.11 mg/mL), and the clean water was served as the control treatment. During the experiment, the entire aqueous solution was renewed every 24 h to maintain ZIF-8 concentration and water quality. The number of dead goldfish in each basin was recorded after 120 h and calculate the mortality rate. The judgment standard of death

status was no breathing or movement by touching the tail.

The soil-mixing bioassay method[9] was used to assess the toxic effects of ZIF-8 on earthworms (*Aporrectodea caliginosa*). At the start of the experiment, earthworms were acclimated in artificial soil under laboratory conditions. For the experiment, 100 g of artificial soil was placed in (6×8×3.5 cm) plastic containers, and different concentrations of ZIF-8 suspensions (0.01, 0.06, and 0.11 mg/mL) were added and thoroughly mixed into the soil. Deionized water was used as a control. Ten healthy adult earthworms were introduced into each soil group and cultured for 5 days. During the experiment, soil moisture (35 ± 2%) was maintained, and the environmental temperature was controlled between 20-25°C. After the cultivation period, the survival rate of the earthworms was recorded to evaluate the impact of ZIF-8 on the earthworms.

Impact of BIS@ZIF-8 on the taxonomic abundance of microbial community

Several soil samples were obtained from the rice experimental field of Fujian Agriculture and Forestry University. The soil samples were treated with different materials (BIS@ZIF-8, ZIF-8, and BIS) for 1 day, and water treatment was used as the control. Then, 5 g of soil samples were randomly weighed and stored on dry ice and sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd for sequencing. The bacterial 16S rRNA gene (V3–V4 regions) was amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCT AAT-3′) by a thermocycler polymerase chain reaction (PCR) system. PCR was performed using TransGen AP221-02: TransStart FastPfu DNA Polymerase. 20 μL of the mixture containing 5×FastPfu Buffer (4 μL), 2.5 mM dNTPs (2 μL), 5 μM forward

primer (0.8 μL), (5 μM) reverse primer 0.8 μL, FastPfu Polymerase (0.4 μL), BSA (0.2 μL), Template DNA (10 ng), and ddH_2O to 20 μL were supplemented. PCR cycle conditions were as follows: initial denaturation at 98°C for 3 min, 27 cycles at 95 °C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 45 s, and a final extension at 72°C for 10 min. After amplification, 2% agarose gel was used to extract the PCR products and enable purification by the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, CA). Quantitation was done using QuantiFluor™-ST blue fluorescence quantification system (Promega). The resulting amplicons were loaded on an Illumina MiSeq platform at an equal amount for sequencing.

The PE reads obtained by Miseq sequencing were spliced according to the overlapping relationship. At the same time, the sequence quality was controlled and filtered. After distinguishing the samples, OTU cluster analysis and species taxonomy analysis were carried out. Based on OTU, the diversity index analysis was carried out. Based on the results of the OTU cluster analysis, the diversity index analysis of OTU was carried out.

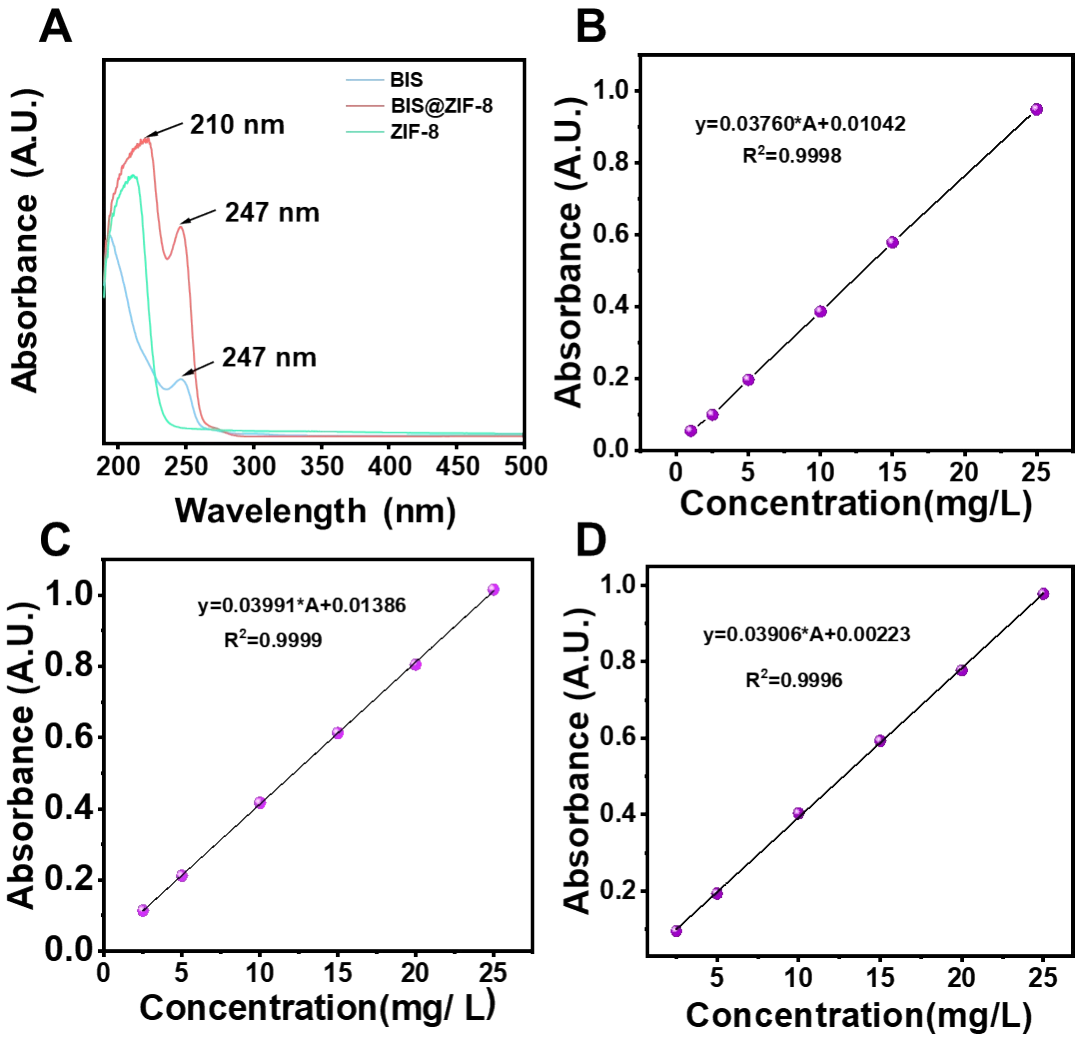

Fig. S1. (A) The ultraviolet scanning spectra of ZIF-8, BIS, and BIS@ZIF-8; Standard curves of BIS in different solutions. (B) distilled water; (C) PBS 5.0; (D) PBS 7.4.

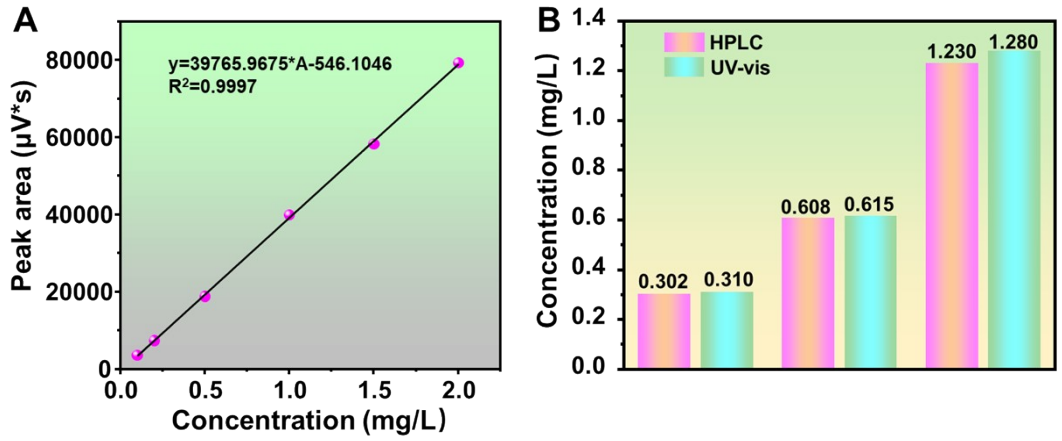

Fig. S2 (A) The standard curve of BIS using HPLC, (B) The concentration of BIS measured using HPLC and UV-vis methods respectively.

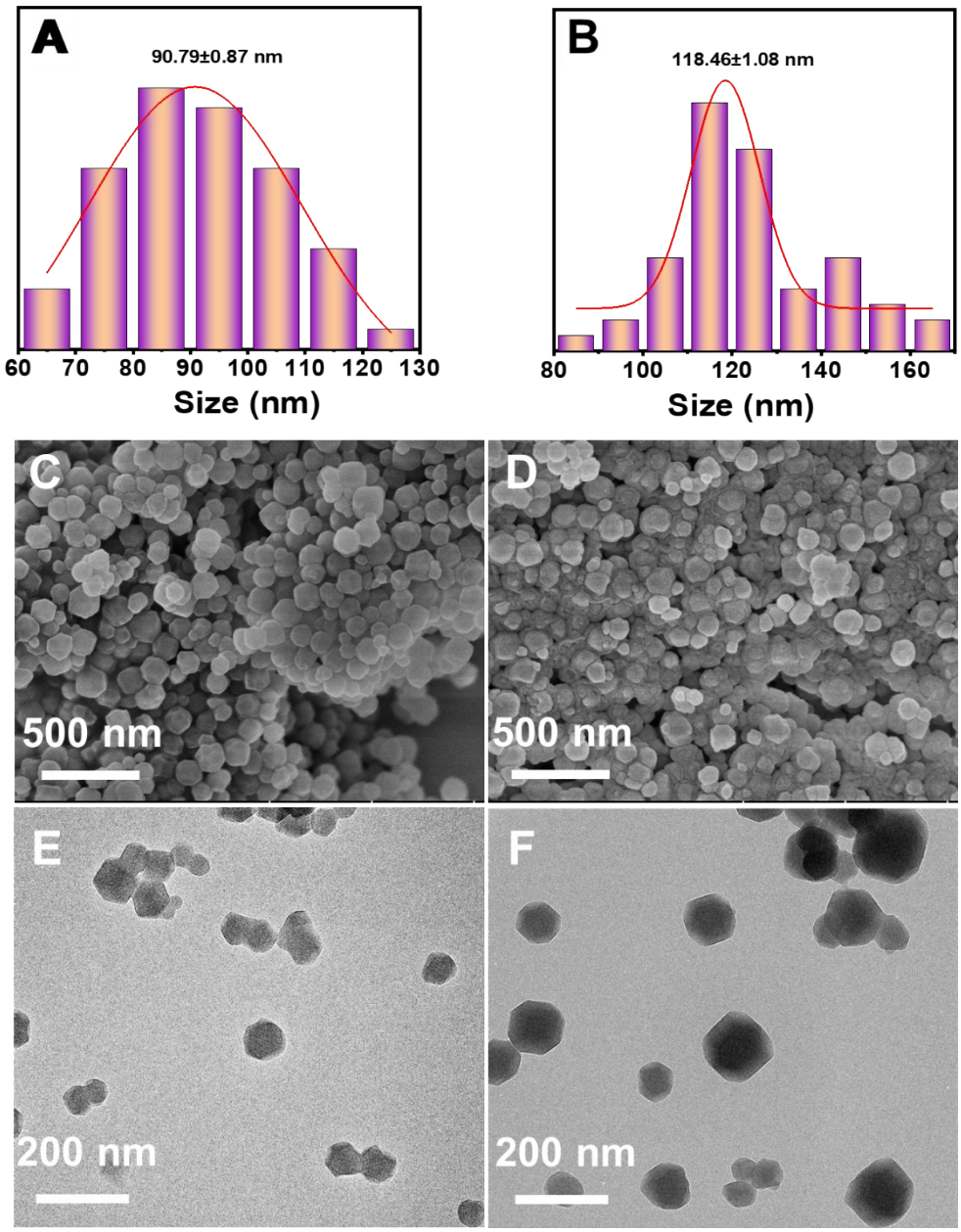

Fig. S3. The particle size and distribution of (A) BIS@ZIF-8 and (B) ZIF-8, SEM images of (C) ZIF-8, (D) BIS@ZIF-8. TEM images of (E) ZIF-8, (F) BIS@ZIF-8.

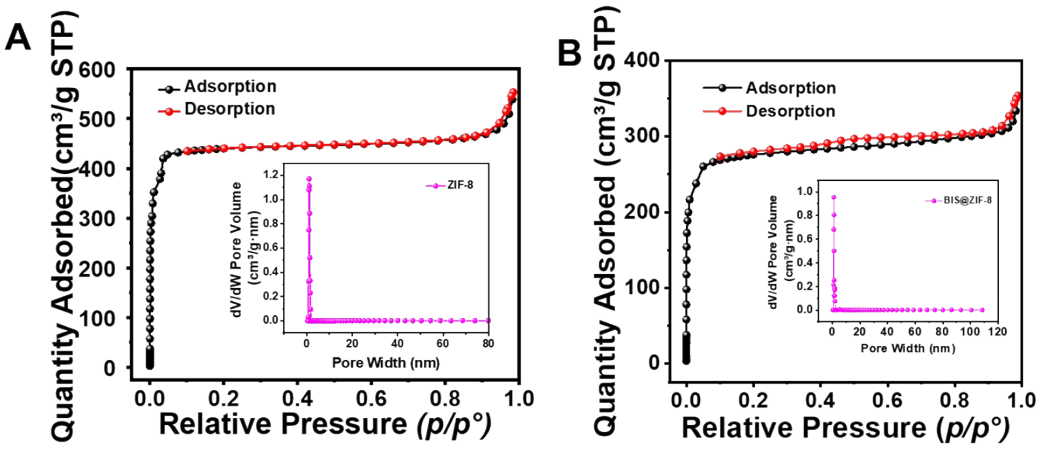

Fig. S4. Nitrogen adsorption−desorption isotherms of (A) ZIF-8, (B) BIS@ZIF-8.

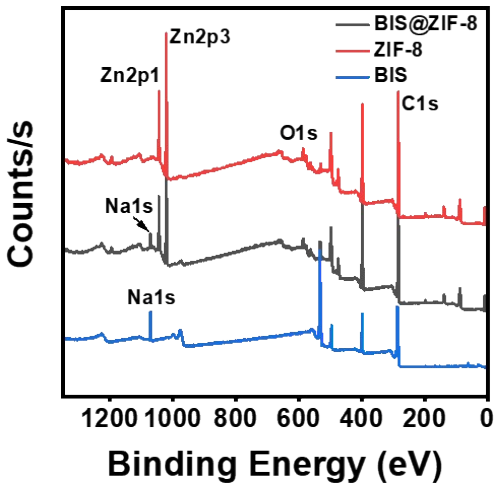

Fig. S5. XPS survey spectra of ZIF-8, BIS and BIS@ZIF-8.

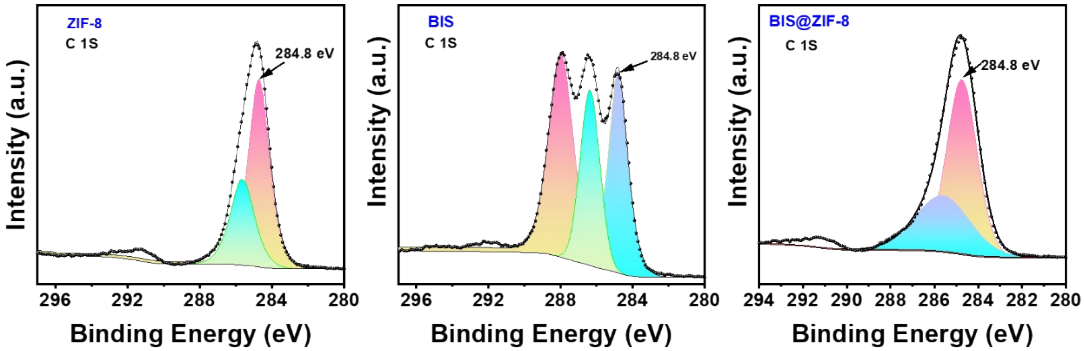

Fig. S6. C1s XPS spectra of ZIF-8, BIS and BIS@ZIF-8 for XPS baseline correction.

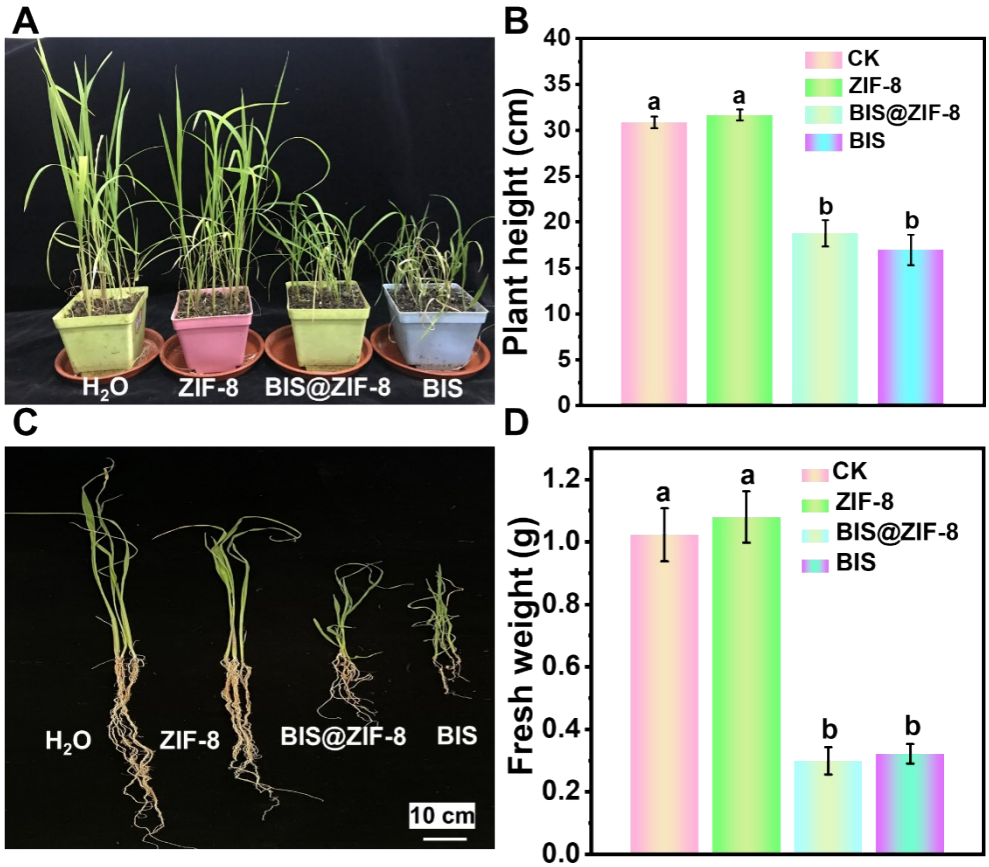

Fig. S7. (A-D) Digital photographs, the plant height, root length, and fresh weight of barnyard grass treated with deionized water, ZIF-8, BIS@ZIF-8, and 0.1 mg/mL BIS after 15 days, respectively. Note: different lowercase letters indicate significant differences ($P< 0.05$)

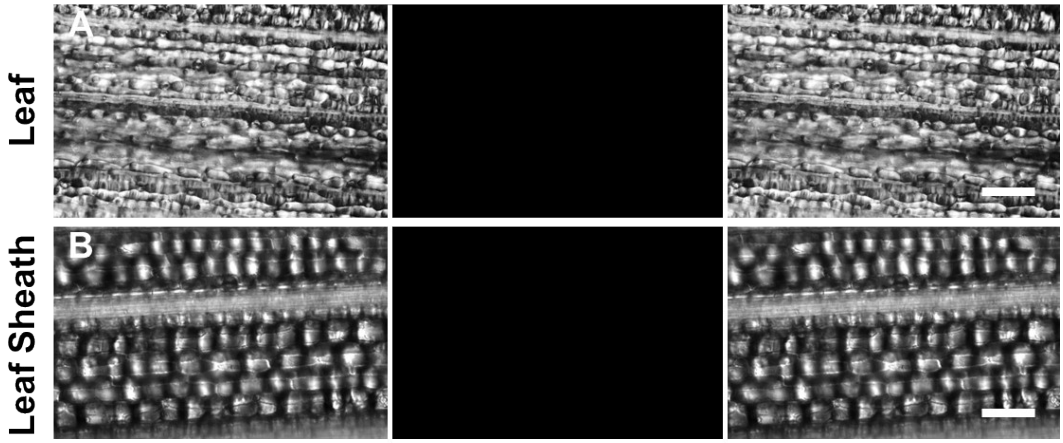

Fig. S8. Observation of barnyardgrass morphology by laser confocal microscope.

(A) Barnyard grass leaves and (B)sheaths treated with water

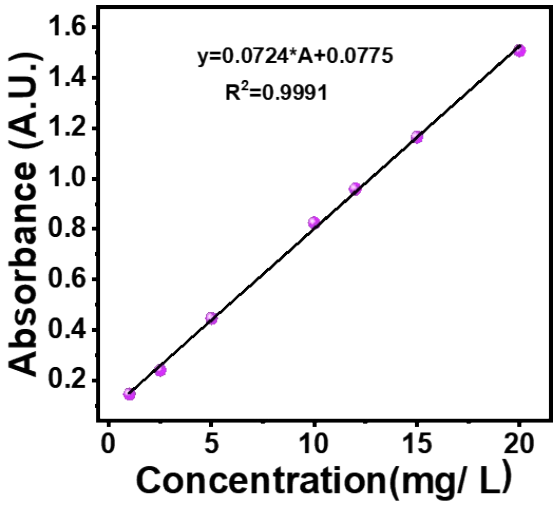

Fig. S9. Standard curves of 2-methyl-imidazole

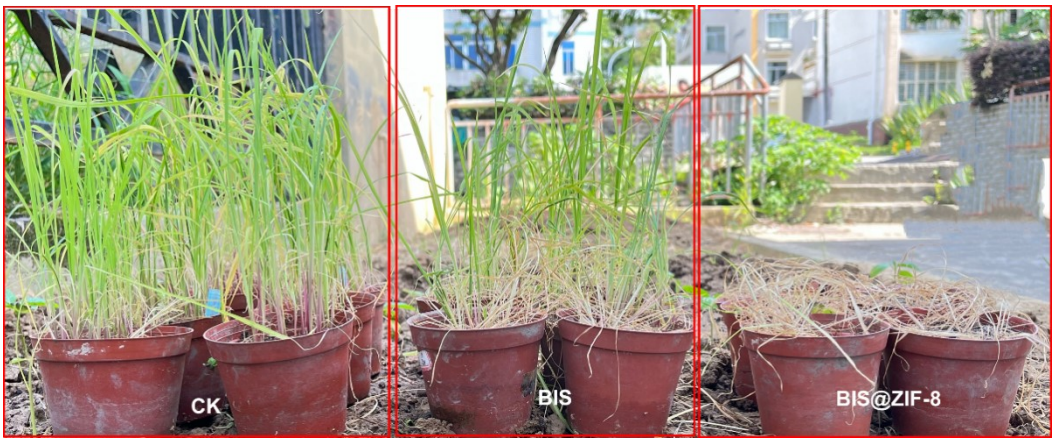

Fig. S10. Field evaluation of the herbicidal activity of BIS@ZIF-8 against barnyard grass (barnyard grass was treated with BIS and BIS @ ZIF-8, respectively, and cultured under field conditions for 10 days.)

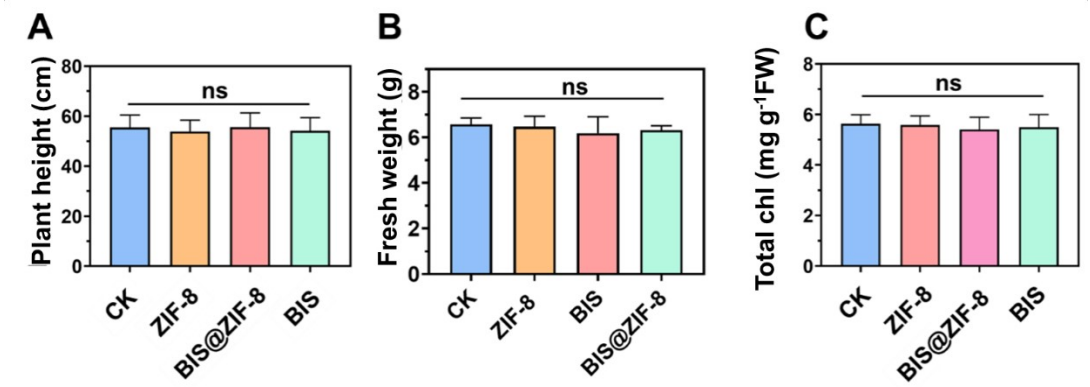

Fig. S11. (A) The plant height, (B) fresh weight and (C) total chlorophyll content of rice after 10 days of different treatments (deionized water, ZIF-8, BIS@ZIF-8, and 0.1mg/mL BIS).

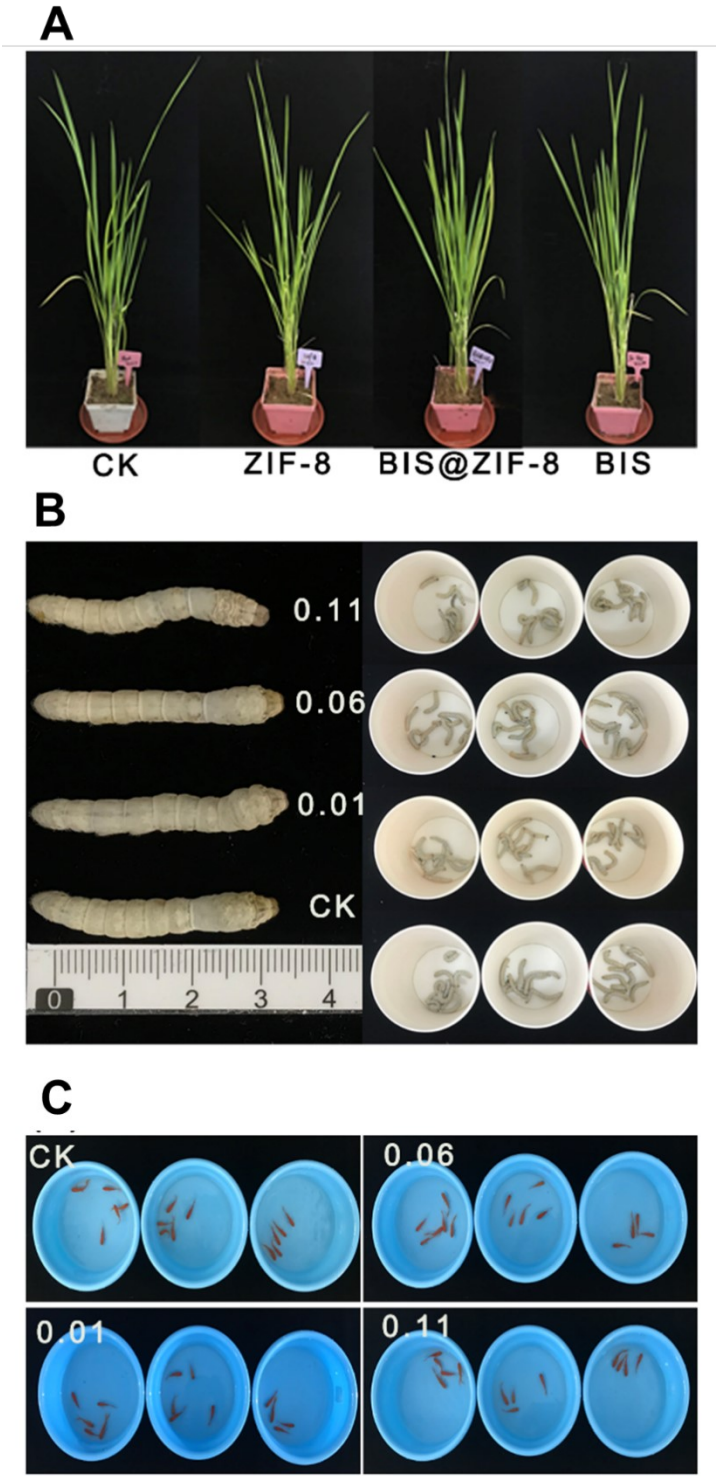

Fig. S12. Digital biosafety images of rice (A), silkworms (B), and goldfish (C) with various concentrations of BIS@ZIF-8 and ZIF-8.

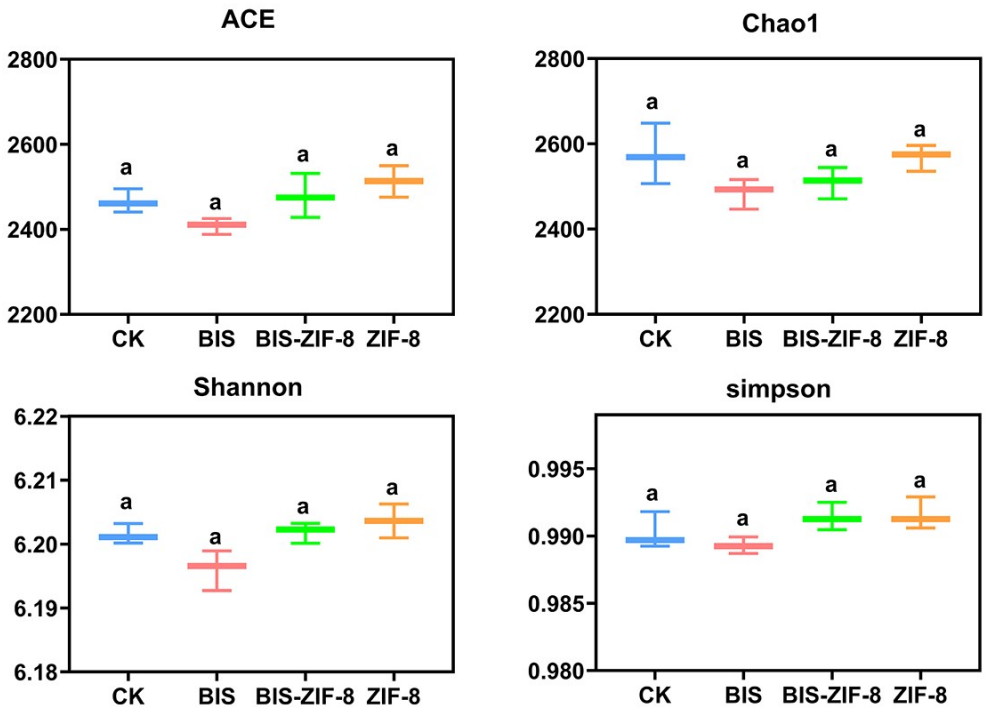

Fig. S13 Analyze the impact of ZIF-8 on the composition of soil microbial communities based on Alpha diversity indices (ACE, Chao1, Shannon, and Simpson).

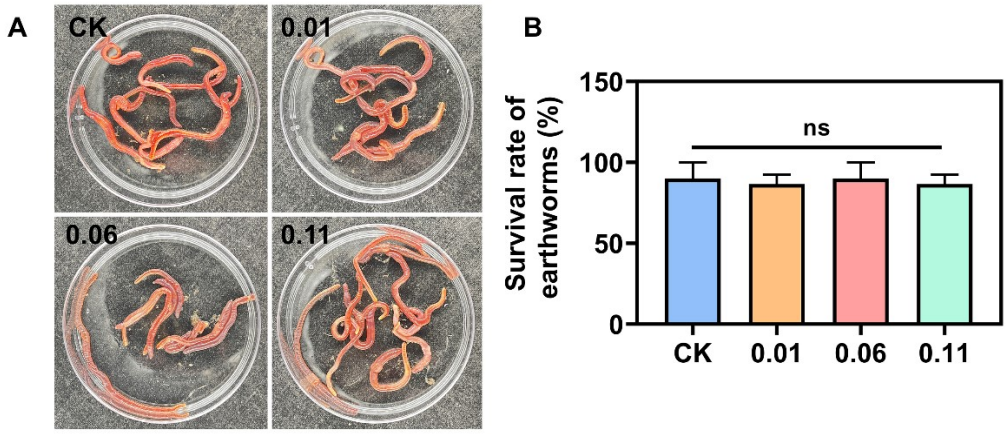

Fig. S14 (A) The image of earthworms after 5 days of treatment. (B) The survival rate of earthworms treated with the different concentrations of ZIF-8 by continuous feeding for 5 days.

Table S1. BET surface area, average particle size and pore volume of ZIF-8 and BIS@ZIF-8.

Sample	BET surface area (m^2/g)	Average particle size (nm)	pore volume (cm^3/g)
ZIF-8	1451.08	4.13	0.67
BIS@ZIF-8	936.97	6.40	0.41

Table S2 Weather conditions in Fuzhou City, Fujian Province, China

Date	Maximum/ Minimum air temperature	Weather	Ultraviolet index	Humidity	Rainfall amount (mm)
2023-06-23	29°C /25°C	light rain	3	98%	3.6
2023-06-24	32°C /25°C	cloudy	5	96%	0
2023-06-25	35°C /25°C	cloudy	7	86%	0
2023-06-26	35°C /25°C	cloudy	6	90%	0
2023-06-27	35°C /26°C	cloudy	11	88%	0
2023-06-28	36°C 27°C	cloudy	13	89%	0
2023-06-29	36°C /27°C	cloudy	6	91%	2.5
2023-06-30	36°C /27°C	cloudy	6	90%	0
2023-07-01	35°C /28°C	cloudy	6	90%	1.0
2023-07-02	35°C /27°C	cloudy	8	90%	0
2023-07-03	35°C /27°C	cloudy	7	88%	0

(Note: Ultraviolet index is expressed as a number from 0 to 15. The weather information sources come from: https://www.tianqishi.com/fuzhou/20230703.html)

References

1 W. L. Liang, Z. G. Xie, J. L. Cheng, D. Xiao, Q. Xiong, Q. Wang, J. Zhao and W. Gui, A light-triggered pH-responsive metal-organic framework for smart delivery of fungicide to control *Sclerotinia* diseases of oilseed rape, *ACS Nano*, 2021, **15**, 6987-6997.

2 J. Tang, X. Tong, Y. Chen, Y. Wu, Z. Zheng, A. B. Kayitmazer, A. Ahmad, N. Ramzan, J. Yang, Q. Huang and Y. Xu, Deposition and water repelling of temperature-responsive nanopesticides on leaves, *Nat. Commun.*, 2023, **14**, 6401.

3 C. Xu, L. Cao, T. Liu, H. Chen and Y. Li, pH-responsive copper-doped ZIF-8 MOF nanoparticles for enhancing the delivery and translocation of pesticides in wheat plants, *Environ. Sci-Nano*, 2023, **10**, 2578-2590.

4 M. Taheri and T. Tsuzuki, Photo-accelerated hydrolysis of metal organic framework ZIF-8, *ACS Materials Letters*, 2021, **3**, 255-260.

5 M. Taheri, D. Ashok, T. Sen, T. G. Enge, N. K. Verma, A. Tricoli, A. Lowe, D. R. Nisbet and T. Tsuzuki, Stability of ZIF-8 nanopowders in bacterial culture media and its implication for antibacterial properties, *Chem. Eng. J.*, 2021, **413**, 127511-127521.

6 J. Liu, M. Hasanuzzaman, H. Wen, J. Zhang, T. Peng, H. Sun and Q. Zhao, High temperature and drought stress cause abscisic acid and reactive oxygen species accumulation and suppress seed germination growth in rice, *Protoplasma*, 2019, **256**, 1217-1227.

7 T. Ahmed, M. Noman, H. Jiang, M. Shahid, C. Ma, Z. Wu, M. M. Nazir, M. A. Ali, J. C. White, J. Chen and B. Li, Bioengineered chitosan-iron nanocomposite controls bacterial leaf blight disease by modulating plant defense response and nutritional status of rice (*Oryza sativa* L.), *Nano Today*, 2022, **45**, 101547.

8 E. A. Goncharuk, M. Y. Zubova, T. L. Nechaeva, V. V. Kazantseva, A. A. Gulevich, E. N. Baranova, P. V. Lapshin, V. M. Katanskaya, M. A. Aksenova and N. V. Zagoskina, Effects of hydrogen peroxide on in vitro cultures of tea (*Camellia sinensis* L.) grown in the dark and in the light: morphology, content of malondialdehyde, and accumulation of various polyphenols, *Molecules*, 2022, **27**, 6674.

9 S. A. A. Abdel-Raheem, M. R. Fouad, M. A. Gad, A. M. Kamal El-Dean and M. S. Tolba, Environmentally green synthesis and characterization of some novel bioactive pyrimidines with excellent bioefficacy and safety profile towards soil organisms, *Journal of Environmental Chemical Engineering*, 2023, **11**, 110839.