**Supplementary Materials**

**Size-Dependent Disease Resistance Enhancement of Hollow Mesoporous Silica Nanoparticles in Cowpea Plant Involved in Salicylic Acid Mediated Systemic Acquired Resistance for Fusarium Wilt Control**

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**1 Synthesis of HMSNs–406 and HMSNs–96**

1.1 Synthesis of sSiO2

The solid core silica nanospheres of 50 nm (sSiO2–50) was preared by the existing methods [1, 2] with some modifications. For details, 10 mL of tetraethyl orthosilicate (TEOS) was added to an reaction mixture containing 20 mL of ultrapure water, 81 mL of anhydrous ethanol, and 2.93 mL of NH3-H2O (25-28%) as the alkaline solvent at 70°C. Hydrolysis and polycondensation of TEOS for 3 h yielded the target particles with a particle size of 50 nm. Then, sSiO2–50 were separated by centrifugation (15000 × g, 15 min) and washed three times with ultrapure water. After the last washing, the system was purified by dialysis with distilled water, and the target particles of sSiO2–50 were obtained after centrifugation and drying.

Synthesis of sSiO2–250 was carried out by varying the inputs of raw materials and reaction time [1]: TEOS (3 mL) was rapidly added to a mixed system of ethanol (37 mL), deionised water (5 mL) and ammonia solution (25-28%, 1.6 mL). The system was stirred and reacted at room temperature for 1 h to produce a suspension of white colloidal silica nanoparticles. The sSiO2–250 were separated from the suspension by centrifugation and washed with deionised water and ethanol, and the target silica nanoparticles with a particle size of 250 nm were obtained after drying.

1.2 Synthesis of HMSNs–406 and HMSNs–96

HMSNs-96 and HMSNs-406 were synthesized according to previously describe mehtods [1, 2] using sSiO2–50 and sSiO2–250 as the cores, respectively. In detail, sSiO2 (200 mg) was ultrasonically dispersed in 40 mL of deionised water, and then the system was added into a mixture of CTAB (300 mg), deionised water (60 mL), ethanol (60 mL), and ammonia (1.1 mL) at room temperature. The mixture was stirred for 0.5 h and then 0.5 mL of TEOS was added to the system and the reaction was continued for another 6 h. After the reaction, the solid particles of sSiO2@CTAB/SiO2 was centrifuged and re-dispersed by ultrasonication in 40 mL of deionised water, and 848 mg of Na2CO3 was added to the system and the reaction was stirred for another 10 h at 50°C. The obtained particles was then washed with ethanol and water, and dried. The resulting solid was placed in a muffle furnace and heated to 550°C at a ramping rate of 1.5°C/min, and then kept for 6 h to remove the CTAB surfactant to obtain the target HMSNs–406 and HMSNs–96.

**2 Synthesis of HMSNs–19**

HMNSs**–**19 was prepared according to a previously reported method [3] with modifications. Pluronic F108 (1 g) and HCl (5 mL) were dissolved in deionised water (25 mL) and stirred vigorously to form a homogeneous system. After Pluronic F108 was completely dissolved, homotrimethylbenzene (0.8 g) was added to the above system and stirred continuously (1000 rpm) at 25°C for 3 h. Then, TEOS (1.0 g) was added dropwise to the reaction system at a dropwise acceleration rate of 0.1 mL/min under stirring. After the reaction was continued for 5 h with stirring, dimethyldimethoxysilane (0.4 g) was added dropwise at a rate of 0.1 mL/min, and the reaction was continued for 36 h. The obtained milky-white system was dialysed for 48 h to remove the hydrochloric acid, and then lyophilised in a vacuum lyophiliser. The resulting solid pellet was dispersed in 30 mL of ethanol-HCl (v/v, 29:1) mixture and the system was refluxed for 12 h to remove the surfactant, and the process was repeated twice. Afterwards, the solid product was collected by centrifugation (10,000 rpm, 15 min) and washed repeatedly with ethanol to completely remove PluronicF108 and HCl. Finally, the solid particles after template removal were dried under vacuum at 60°C overnight to obtain the target HMNSs**–**19.

**3 Mycelial growth inhibition test**

The direct suppression of three HMSNs against *F. oxysporum* f. sp. *phaseoli* (FOP) was determined by the previously reported agar dilution method [4] using the mycelial growth inhibition as the indicator. Briefly, stocks of three HMSNs were prepared by ultrasonic dispersion of them in sterile water to gain the final concentrations of 1, 10 and 20 mg/mL, respectively. Each stock solution (5 mL) was added to molten PDA (50 mL) at temperatures below 50°C. After sufficient mixing, the mixture was poured immediately into 90-mm Petri dishes to form plates of 2-3 mm thickness with the HMSNs concentration of 100, 1000 or 2000 mg/L. PDA plates supplemented with sterile water served as the control. Afterwards, a 5-mm diameter mycelial disc from the actively growing colony front of FOP was then placed in the center of each plate with the inoculum-side down. All the plates were then incubated in the dark at a constant temperature of 25 °C for 7 days. Each treatment was performed in triplicate. The mycelial growth diameters were measured and subsequently converted into an inhibition rates according to the following equation **S1**:

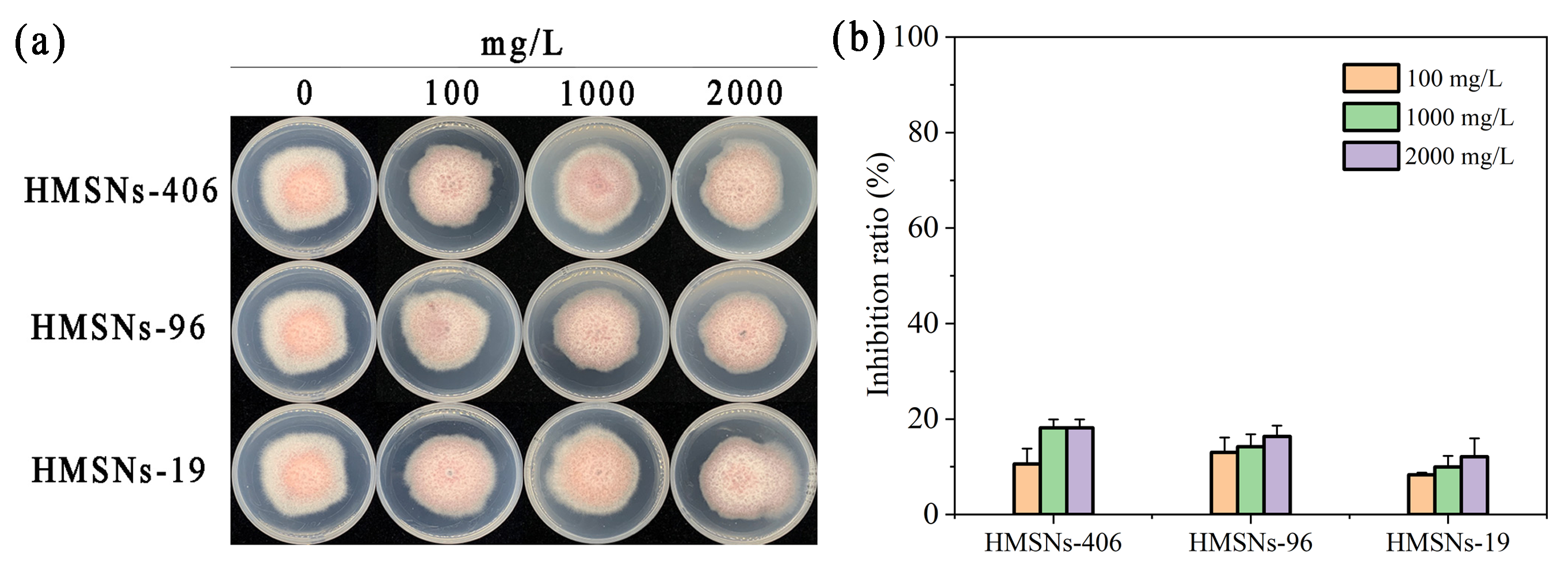
inhibition rate (%) = [(Dc − Dt)/(Dc − 0.5)] × 100 (S1)

where Dc and Dt indicate the mycelial growth diameters of the control and treatment groups, respectively.

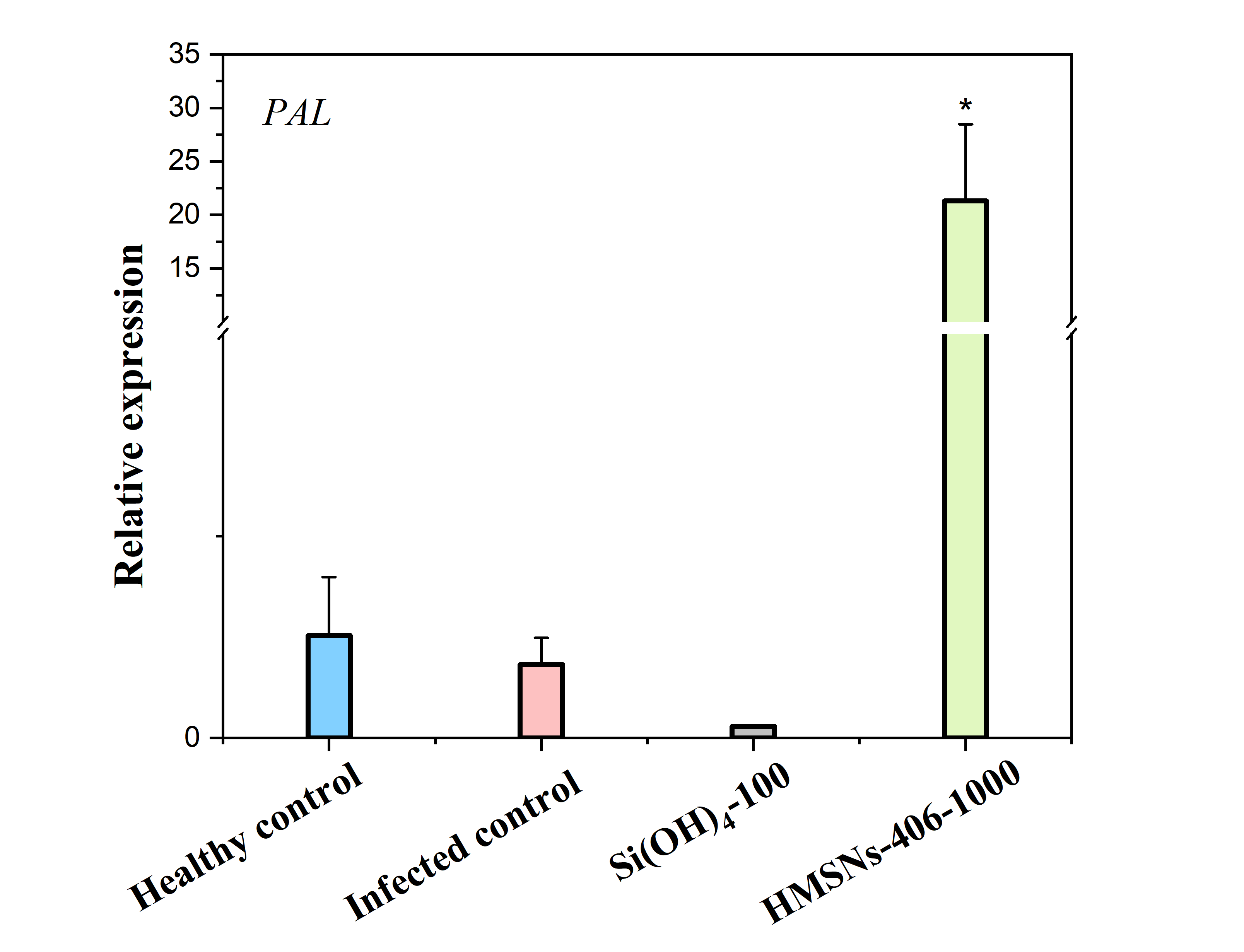
**4 Salicylic acid (SA) Measurement**

SA content of free and conjugated forms in cowpea roots was measured according to a previously described ultra-performance liquid chromatography (UPLC) method [5, 6] with some modifications. Briefly, 0.2 g of each fresh root sample was grounded under liquid nitrigen to obtain the root homogenate. After homogenization, 1 mL of a precooled methanol aqueous solution (v:v, 9:1) was added to extract total SA at 4ºC overnight. The mixture was then centrifuged to (10 min, 8000 g) to obtain the supernate and precipitate. The precipitate was extracted again by 0.5 mL of the precooled methanol aqueous solution. After centrifugation, the second supernate was obtained and merged with the first supernate. The total supernate was concentrated by the vacuum evaporation method at 40ºC to remove methanol. After that, 20 μL of a trichloroacetic acid aqueous solution (1 mg/mL) was added into the above concentrate. After sufficient mixing by shaking, 1 mL of an aethyl acetate-cyclohexane mixture (v:v, 1:1) was used to extract the free SA for twice. The total organic phase was then dried with nitrogen blowing and dissolved by methanol for UPLC analysis. The remaining water phase was mixed with 0.5 mL of a HCl solution (2 mol/L) and the conjugated SA in the system was hydrolyzed at 80ºC for 1 h to become the free form. Then, the SA was extracted by 1 mL of an aethyl acetate-cyclohexane mixture(v:v, 1:1) for twice. The obtained organic phase was then dried with nitrogen blowing and dissolved by methanol for UPLC analysis.

Quantitative determination of overall SA of free and conjugated forms in cowpea roots was performed on a Rigol L3000 UPLC (Bejing, China) with fluorescence detection. A Compass-C18 column (250 mm×4.6 mm, 5 μm particle size) was used for SA separation at 35ºC, using a methanol-water mixture (3:2, v/v) supplemented with 0.4% acetic acid as the elution. The injection volume was 10 μL and the flow rate was 0.8 mL/min. The excitation wavelength was set as 294 nm and the fluorescence signal was collected at 426 nm. A SA standard curve was established using this method for quantitative determination of SA in samples. Total SA was calculated by the sum of free and conjugated SA. Each test was repeated three times.



**Fig. S1 Toxicity of three HMSNs to *Fusarium oxysporum* f. sp. *phaseolus* (FOP) *in vitro*. (a) mycelial growth phenotype; and (b) In-dish inhibition activity of three HMSNs.**



**Fig. S2 RT-qPCR analysis of the gene expression of an antioxidant defence-related enzyme gene *PAL* in cowpea roots. Root samples were collected 29 days after FOP infection. *EF1b* was used as the reference gene. The healthy control represents cowpea plants growing in the noninfected soil and treated with water. Other treatments represent cowpea plants growing in the infected soil and foliarly treated with water (the infected control), Si(OH)4 (100 mg/L) or HMSNs–406 (1000 mg/L). The error bars are averages and standard deviations of three replicates. Asterisks (\*) represent significant differences as compared with the infected controls using the one-way ANOVA mode for significance testing with Dunnett’s multiple comparisons test at *P* < 0.05.**

**Tab. S1 Zeta potential, PDI, and** **average particle size of three HMSNs**

|  |  |  |  |
| --- | --- | --- | --- |
| **HMSNsa** | **Z-Averageb (nm)** | **PDIc** | **Zetad (mV)** |
| HMSNs–19 | 59.26±0.19 | 0.14±0.01 | -19.6±0.3 |
| HMSNs–96 | 202.00±0.60 | 0.36±0.02 | -33.0±1.0 |
| HMSNs–406 | 690.70±12.00 | 0.32±0.01 | -31.0±0.5 |

aHMSNs: Hollow mesoporous silica nanoparticles; bZ-Average: Average particle size; cPDI: Polydis persity index; dZeta: Surface potential. Averages ± standard deviations.

**Tab. S2 Standards of disease severity grading for Fusarium wilt in cowpea plant**

|  |  |  |
| --- | --- | --- |
| **Scale value** | **External leaf symptoms** | **Internal vascular symptoms** |
| 0 | asymptomatic plants | no vascular browning |
| 1 | slight epinastic response and mild chlorosis of the lower third of the plant | up to 25% vascular browning |
| 2 | epinastic response in between 30%–50% of the leaves and moderate chlorosis in mature leaves | 26–50% vascular browning |
| 3 | epinastic response in between 60%–80% of the leaves and moderate chlorosis in the middle third | 51%–75% vascular browning |
| 4 | epinastic response in all the leaves of the plant, severe chlorosis and defoliation, dead plant | >75% vascular browning |

**Tab. S3 Primers for qRT-PCR assay**

|  |  |
| --- | --- |
| **Primers** | **Sequence (5’-3’)** |
| PR1-F | ACTACAACTACGCTGCGAACAC |
| PR1-R | GTTACACCTCACTTTGGCACATC |
| PR5-F | GTGTTCATCACAAGCGGCAT |
| PR5-R | GGGAAGCACCTGGAGTCAAT |
| NPR1-F  NPR1-R  EF1b-F  EF1b-R  PAL-F  PAL-R  PPO-F  PPO-R | TGCTCGGAAGTGTTGGATAAG  GAAATCCCAGAGCGGCTAAA  CCACTGCTGAAGAAGATGATGATG  AAGGACAGAAGACTTGCCACTC  GTTTGTGAGGGAGGAGTTAGAG  ATGGGAGACCCTTTCCAATC  GCTCCTCATAACACGGTTCATA  GTCCTTTCTTCCTTCTCCCAAT |

**Tab. S4 Ampliﬁcation program for qRT-PCR assay**

|  |  |
| --- | --- |
| **System** | **Procedure** |
| SYBR® Premix Ex TaqTM II 5.0 µL  PCR Forward Primer (10 um) 0.3 µL  PCR Reverse Primer (10 um) 0.3 µL  cDNA solution 1.0 µL  Easy dilution (for Real Time) 3.4 µL | Predegeneration at 95ºC for 10 min  Cycle nitiation:  Degeneration at 95ºC for 15 s  Renaturation 60ºC for 60 s  40 cycles |

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