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Supplementary Information

Chitosan-silica nanocomposites induced resistance in faba bean plants against aphids *(Acyrthosiphon pisum)*

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Supplementary methods

Text S1. Measurement of leaf oxidative stress and antioxidant enzyme activity

H_2O_2 concentration was determined according to the previously reported method with a slight modification.[1] Briefly, 100 mg fresh faba bean leaves were homogenized in an ice bath with 1.5 mL 0.1% (w/v) trichloroacetic acid, and then centrifuged at 12000 rpm (16980 xg) at 4 °C for 15 min and the supernatant collected. Afterward, 0.5 mL of the supernatant was added to 0.5 mL of potassium phosphate buffer (10 mM, pH 7), and the absorbance of the supernatant was measured using Microplate Reader (Varioskan Lux, America) at 390 nm.

Superoxide anion ($O_2^{\bullet-}$) content was analyzed according to the previously reported method with a slight modification.[2] Briefly, fresh faba bean leaf (100 mg) was ground in 1.5 mL cold potassium phosphate buffer (pH 7.8) and the mixtures were centrifugation at 12000 rpm (16980 xg) at 4 °C for 10 min. The supernatant (1 mL) was added to phosphate buffer (0.9 mL) and hydroxylamine hydrochloride (0.1 mL) and the mixtures reacted for 30 min at 25 °C, then 17 mmol L^{-1} sulfanilic acid (1 mL) and 7 mmol L^{-1} α-naphthylamine (1 mL) were added the reaction mixture and the solution was continues to react at 25 °C for 20min. The absorbance of the supernatant was measured using Microplate Reader (Varioskan Lux, America) at 530 nm.

Lipid peroxidation was estimated in terms of malondialdehyde (MDA) concentrations by previous studies with a slight modification.[3, 4] Fresh faba bean leaves (100 mg) were homogenized in 1.5 mL of chilled 80% ethanol, and centrifuged at 12000 rpm (16980 xg) at 4 °C for 20 min. The extract (1 mL) was then mixed with 4 mL aqueous solution of 20% trichloroacetic acid and the aqueous solution of 0.67% 2-thiobarbituric acid, incubated at 90 °C for 30 min. The reaction stopped by putting the tubes in an ice bath and then centrifuged at 12000 rpm (16980 xg) for 30 min. The absorbance of the supernatant was recorded using Microplate Reader (Varioskan Lux, America) at 440, 532 and 600 nm.

The activity of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in faba bean leaves were determined according to a previous study with minor

modification.[5] Briefly, Fresh leaves (100 mg) were ground in 50 mM pre-cooled PBS (pH 7.8) containing 1% polyvinylpyrrolidone. The homogeneous was centrifuged 12000 rpm (16980 xg), 4 °C, 20 min), and the supernatants were enzyme extracts. SOD activity was determined by photochemical NBT method in 3 mL reaction mixture. The reaction mixture, including 50 mM PBS (pH 7.8), 75 μM NBT, 10 μM EDTA-Na_2, 13.05 mM methionine, 2 μM riboflavin, and 100 μL enzyme extracts, was shaken and exposed to light for 20 min. Two hundred μL of the reaction mixture was transferred to a multifunctional microplate and measured using Microplate Reader (Varioskan Lux, America) at 560 nm using a spectrophotometer. One unit of SOD is defined as being present in the extract, which can inhibit by 50% of NBT photoreduction. POD activity was measured with guaiacol as the substrate. The absorbance of 200 μL reaction mixture (20 mM potassium phosphate buffer (pH 6.0), 0.56% guaiacol, 0.01% H_2O_2, and 6.67 μL enzyme extracts) was recorded using Microplate Reader (Varioskan Lux, America) for 3 min at 470 nm. The increase of 0.01 of OD value per min was defined as one unit of POD. For CAT activity, the absorbance of 200 μL of the reaction mixture (15 mM phosphate buffer (pH 7.0), 0.05% H_2O_2, and 6.67 μL enzyme extracts) was recorded using Microplate Reader (Varioskan Lux, America) for 3 min at 240 nm. The reduction of 0.01 of OD value per min was used as one unit of CAT

Text S2. Measurement of leaf calcium ion fluxes by NMT

The net fluxes of Ca^{2+} in mesophyll cells was measured using Non-invasive Micro-test Technique system (NMT100S-SIM-XY, Xuyue, Beijing, China) as previously described with minor modifications.[6, 7] Briefly, potassium chloride (0.1 M) was used for silver chloride, and then pre-pulled and moisturized microelectrodes (4-5 μm, XY-DJ-01) were filled with the electrolyte solution (Ca^{2+}: 100 mM $CaCl_{2)}$ to a length of about 1.0 cm followed by filling of selective liquid ion-exchange cocktails (LIXs, Ca^{2+}: XY-SJ- Ca, 40-50 μm, Younger, USA). The microelectrodes were connected to the NMT system with a silver chloride wire and then calibrated in solutions with 0.1 and 0.05 mM $CaCl_2$ for Ca^{2+} flux (Nernstian slope 28 ± 5 mV per

decade). All measurements were recorded at 75 times. Each treatment was measured using five biological replicates. Ca^{2+} fluxes were measured away from about 1-2 μm from the cell surface. The final flux values are reported as the mean of five individual plants per treatment.

Text S3. Determination of leaf phytohormones by LC-MS/MS

The content of phytohormones (JA, and SA,) in faba bean leaves were determined according to a previous method[7] with a slight modification. Briefly, 100 mg leaves were ground in liquid nitrogen and transferred into 1 mL of cold extraction solution (ethyl acetate containing 10 $\mu g \cdot mL^{-1}$ BHT-butylhydroxytoluene). The mixture was vortexed for 15 min, sonicated in an ice bath for 15 min, centrifuged at 12000 rpm (16980 xg) for 10 min at 4 °C, and then transferred the supernatant to a new centrifuge tube. The solution was gently blown dry with nitrogen, reconstituted with 200 μL of 70% methanol, vortexed for 5 min, sonicated in an ice bath for 5 min, and centrifuged at 12000 rpm (16980 xg) at 4 °C for 10 min. Finally, pipette 100 μL of the supernatant into the input sample for LC-MS/MS using an UHPLC system (Vanquish, Germany). A standard solution of JA, and SA (Sigma-Aldrich, Switzerland) was measured every ten samples for quality control. The mobile phase A (H_2O, 0.01% formic acid) and B (acetonitrile, 0.01% formic acid formic acid in acetonitrile) were used for the elution gradient: 0 min, 5 % B; 1.5 min, 5 % B; 9 min, 70 % B; 10 min, 70 % B; 10.1 min, 5 % B; 15 min, 5 % B. The flow rate and injection volume were 0.35 mL min^{-1} and 5 μL, respectively. The concentration of phytohormones was conducted by using a calibration equation obtained by linear regression from five calibration points for each analysis. The R^2 value for the JA and SA standard was 0.9992, 0.9984, respectively.

Text S4. LC-MS/MS analysis of faba bean leaf metabolites.

The extraction and identification of faba bean leaf metabolites were according to a previous study with a slight modification.[8] Briefly, faba bean leaves (100 mg) were powdered in liquid nitrogen and transferred to 2 mL tubes, and then added into 1.5

mL of 80% methanol/water with 0.1% formic acid and 0.2 mg/L 2-chloro-L-phenylalanine as internal standard. Subsequently, the mixture was vortexed for 1 min and ultrasonicated for 30 min in ice, and then centrifuged at 4 ℃, 12000 rpm (16980 xg) for 15 min. After that, the supernatant was freeze-dried and re-dissolved in 200 μL solvent of methanol, acetonitrile, and water (4:4:2, v/v/v) followed by centrifuging at 4 ℃, 12000 rpm (16980 xg) for 15 min. Eventually the supernatant was detected analyzed by LC-MS/MS (Thermo Fisher, Germany). The quality control (QC) samples were prepared with a mixture of the same amount of all samples. The mobile phase A (H_2O, 0.1% formic) and B (acetonitrile, 0.1% formic acid) were used for the elution gradient: 0 min, 5 % B; 1.5 min, 5% B; 10 min, 100 % B; 11min 100%B; 11.5 min, 5 % B; 14 min, 5 % B. The injection volume and the flow rate were 5 μL and 0.35 mL min^{-1}, respectively. The parameters of ESI were: sheath gas pressure, 35 arbitrary units; aux gas flow, 15 arbitrary units; sweep gas flow, 0 arbitrary units; capillary temperature, 320 °C; aux gas heater temperature, 350 °C. Spray voltage was 3.5 kV for ESI (positive) and -3.0 kV for ESI (negative).

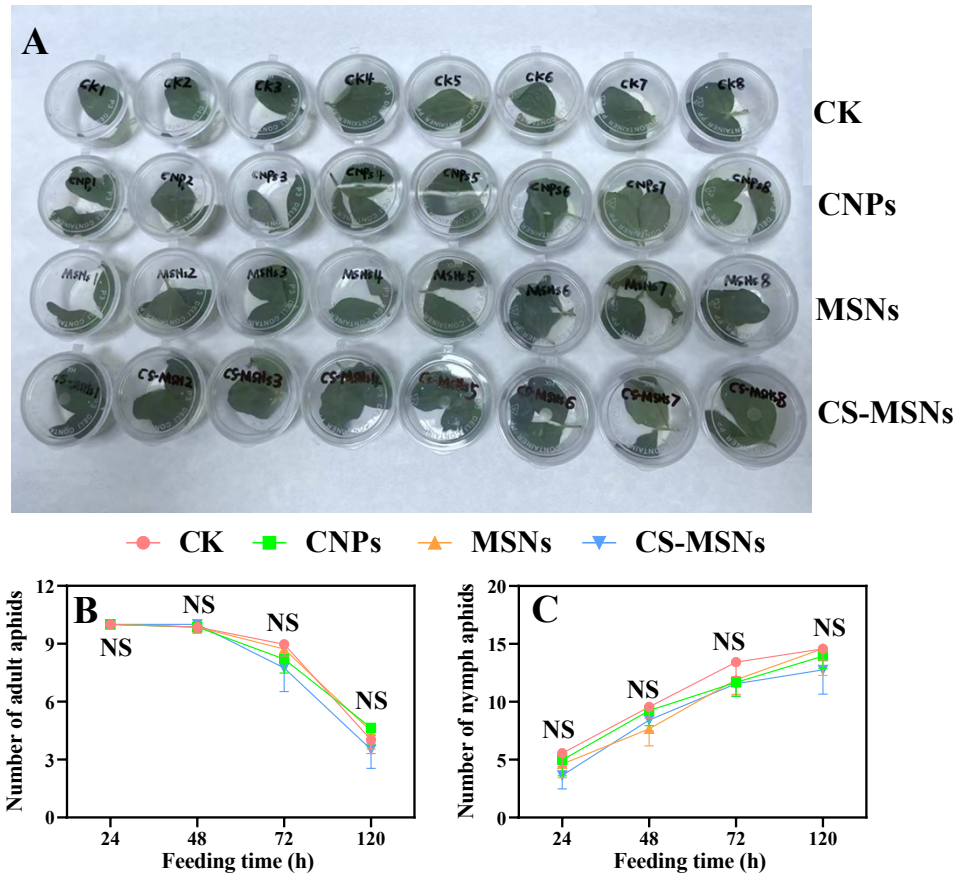

Fig. S1 Feeding bioassay experiment testing the direct entomotoxic effect of 100 mg/L NMs (CNPs, MSNs, CS-MSNs) on aphids after 24, 48, 72 and 120 h, respectively. Feeding bioassay experiment was carried out with fresh faba bean leaves in the box within 120 h feeding period (A). Adult aphid population (B), nymph aphid population (C). NS represents non-significant differences among treatments ($p < 0.05$).

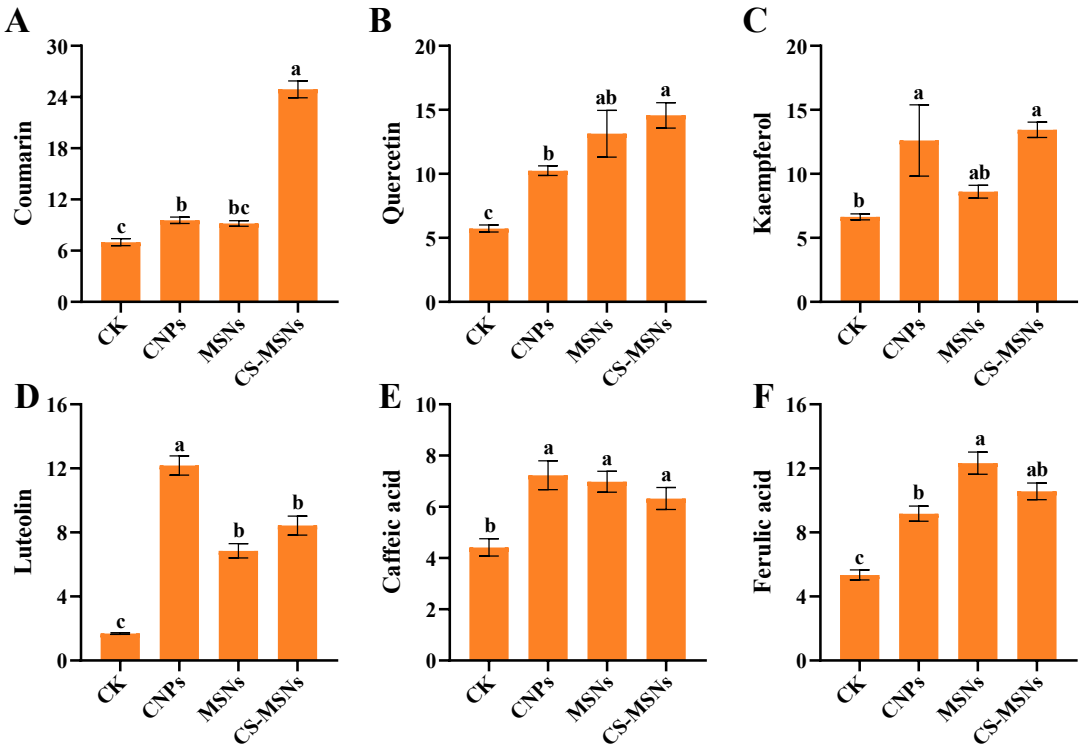

Fig. S2 The relative abundances of metabolites in faba bean plants exposed to NMs in response to aphids. Coumarin (A), Quercetin (B), Kaempferol (C), Luteolin (D), Caffeic acid (E) and Ferulic acid (F) in leaves. Significant differences among treatments are marked with different lowercase letters ($p < 0.05$).

Table S1. Summary table of two-way ANOVA results showing the interactive effects of NM treatment and herbivore treatment with aphids on faba bean plant growth, oxidative stress, antioxidant enzyme, Ca^{2+} and phytohormone traits. The test results are shown with the test statistic F-value and significance levels ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ and $^{NS}p > 0.05$)

Plant traits	NM treatment	Herbivore	NM treatment × Herbivore
Shoot biomass	7.8 ***	2.6 NS	0.3 NS
Root biomass	0.8 NS	7.5 **	0.1 NS
Leaf Chlorophyll	45.9 ***	96.0 ***	1.2 NS
Leaf Pn	15.8 ***	18.2 ***	0.2 NS
Leaf H_2O_2	4.5 ***	61.5 ***	2.2 NS
Leaf $O_2 \cdot^-$	6.3 ***	0.3 NS	2.4 *
Leaf MDA	3.1 *	7.4 **	3.4 **
Leaf SOD	5.2 ***	4.2 *	0.3 NS
Leaf POD	14.9 ***	102.1 ***	1.2 NS
Leaf CAT	12.6 ***	6.7 *	0.0 NS
Leaf Ca^{2+}	329.1 ***	293.8 ***	7.1 ***
Leaf SA	47.5 ***	267.0 ***	4.5 **
Leaf JA	13.3 ***	135.0 ***	4.3 *

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