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RESEARCH ARTICLE

Synergistic incorporation of nano-pesticides into biological control: Excellent biocompatibility with parasitic wasps (*Aphidius colemani*) can achieve efficient aphid control

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Highlights

- Star polycation (SPc) shows negligible toxicity in parasitic wasps (*Aphidius colemani*).
- Parasitic wasps show high tolerance, and the clothianidin/SPc complex causes no adverse impacts.
- Co-application of the clothianidin/SPc complex and parasitoids achieves excellent cooperative pest management.

Abstract

The synergistic use of chemical pesticides and biological agents poses the fundamental challenge of balancing control efficacy with ecological safety. In recent years, nanotechnology has emerged as a promising strategy for improving pesticide performance while reducing pesticide residues and alleviating environmental contamination. Herein, we developed an efficient nano-pesticide based on star polycation (SPc) loaded with clothianidin, which was co-applied with a widely used parasitic wasp (*Aphidius colemani*) to achieve synergistic pest management. SPc at the working concentration displayed no significant impact on the eclosion or survival of parasitic wasps, whereas the oral feeding of SPc at an extremely high concentration significantly up-regulated several genes related to ribosomal protein and energy metabolism, leading to metabolic imbalance and subsequent mortality of the parasitic wasps. The SPc could load clothianidin *via* hydrogen bonding and Van der Waals forces, and this spontaneous complexation achieved a reduction in particle size from 6,554.87 to 467.84 nm. Importantly, the clothianidin/SPc complex exhibited a 16–28% increase in insecticidal activity against green peach aphids (*Myzus persicae*), while showing minimal adverse impacts on the eclosion and parasitism of parasitic wasps. Finally, co-application of the clothianidin/SPc complex with parasitic wasps achieved up to 80% mortality in green peach aphids, with the promising advantages of rapid pest suppression and sustainable control. This study proposes a synergetic pest management strategy based on nano-pesticides and natural enemies, which is beneficial for maintaining long-term agricultural ecological balance.

Keywords: biological control, cooperative control, nano-pesticide, parasitic wasp, pest management

1. Introduction

Chemical pesticides play a crucial role in agricultural development, since they ensure stable global food production by effectively controlling insect pests and plant diseases (Sparks and Bryant 2021; Tudi *et al.* 2021). However, their excessive application has led to a series of ecological concerns, including the acceleration of pest resistance, a decline in non-target biodiversity, and severe environmental pollution (Wang *et al.* 2019; Neale *et al.* 2020; Deng *et al.* 2021). These challenges underscore the urgent need for

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eco-friendly agrochemicals that maintain high efficacy while minimizing negative environmental impacts. To mitigate the ecological risks associated with chemical pesticides, biological control has gained significant attention as an eco-friendly pest management strategy. *Aphidius colemani* Viereck (Hymenoptera: Braconidae) is a widely used parasitic wasp that effectively targets over 41 aphid species, making it one of the most successful biological control agents in greenhouse crops (Stary 1975; Santos *et al.* 2019). When parasitized by *A. colemani*, the aphids undergo physiological changes and eventually form mummies (Stara *et al.* 2011). Despite its advantages, including ease of mass production, high reproductive capacity, and low cost, the effectiveness of *A. colemani* is often influenced by environmental conditions, host density and pesticide residues (Takahashi *et al.* 2005; Colinet *et al.* 2006). In some cases, biological control alone has proven insufficient, as aphid populations can outpace the reproductive rate of parasitoids (Van Driesche *et al.* 2008; Nagasaka *et al.* 2010). Consequently, many growers continue to rely on chemical pesticides to complement their biological control efforts.

The synergistic use of chemical pesticides and natural enemies poses the fundamental challenge of balancing control efficacy with ecological safety. Previous studies have indicated that the parasitism rate declines significantly when the aphid density exceeds twice the population of parasitic wasps, often necessitating the use of chemical pesticides (Helenius 1990; Baggen and Gurr 1998). However, even eco-friendly insecticides can compromise natural enemies due to residual toxicity (Lin *et al.* 2016). Neonicotinoids that act on nicotinic acetylcholine receptors (nAChRs) are widely detected in agricultural environments, and they have been shown to induce neurotoxic effects on non-targets (El Khoury *et al.* 2022). In addition, conventional pesticide formulations suffer from poor dispersion and weak adhesion, resulting in over 90% of the applied chemicals dispersing into the environment (Zhao *et al.* 2018). Therefore, developing pesticide formulations that not only exhibit strong target specificity but also minimize adverse effects on natural enemies is essential.

As a neonicotinoid insecticide, clothianidin exhibits insecticidal activity against a wide range of insect pests. However, its practical application is hindered by issues such as poor water solubility and long environmental persistence (Uneme 2011; Bonmatin *et al.* 2015; Mörtl *et al.* 2020). In recent years, nanotechnology has emerged as a promising strategy for improving pesticide performance (Vishnu *et al.* 2024). Compared to traditional pesticides, nano-pesticides possess several advantages, including reduced particle size, much greater surface area, and enhanced adhesion properties. These attributes contribute to improved pesticide stability, reduced pesticide residues, and less environmental contamination (Iavicoli *et al.* 2017; Adisa *et al.* 2019). For instance, previous studies have demonstrated that nano-chitosan loaded with abamectin significantly enhances its control efficacy against *Spodoptera litura* while reducing pesticide usage by approximately 50% (Li *et al.* 2020). Moreover, nano-silica has been shown to enhance the attraction of natural enemies without interfering with plant growth (Thabet *et al.* 2021). Nevertheless, the biocompatibility of nanomaterials remains a critical issue. The safety and efficacy of nanoparticles are influenced by their physicochemical properties, application methods, and dosages (Zhao *et al.* 2020). Before the application of nano-pesticides associated with natural enemies, assessing their potential impacts on natural enemies is crucial to ensure ecological safety.

In this study, we aimed to develop a clothianidin nano-pesticide based on a star polycation (SPc) nanocarrier, and investigated its synergistic application with *A. colemani*. SPc is positively charged (+20.9 mV) with a particle size of 100.5 nm and molecular weight of about 40,296 Da (Li *et al.* 2019). First, the biological toxicity of SPc was extensively assessed against *A. colemani*, and RNA-seq analysis was conducted to elucidate the lethal mechanism of SPc at an extremely high concentration. Subsequently, the self-assembly mechanism of the clothianidin/SPc complex was investigated using isothermal titration calorimetry (ITC), and its particle size and morphology were examined using dynamic light scattering (DLS) and scanning electron microscopy (SEM), respectively. Then, the insecticidal efficacy of the clothianidin/SPc complex was evaluated against green peach aphids (*Myzus persicae*), a global agricultural pest that can infect over 100 plant species from more than 40 families (Zeng *et al.* 2016). Meanwhile, the potential adverse effects of the clothianidin/SPc complex were assessed on the eclosion and parasitism of *A. colemani*. Finally, the co-application of clothianidin/SPc complex with *A. colemani* was evaluated to examine their synergistic effect. This study provides valuable insights into the potential for a more sustainable and efficient pest management strategy, which can optimize pest control efficacy while maintaining the ecological balance.

2. Materials and methods

2.1. Chemical reagents and insect rearing

Pure clothianidin (≥97%) was obtained from Shanghai Yuanye Biotechnology Co. (China). For SPc synthesis, 2-bromo-2-methylpropionyl bromide and triethylamine were sourced from Heowns BioChem Technologies (China). 2-(Dimethylamino)ethyl methacrylate (DMAEMA) was purchased from Energy Chemical (China). N,N,N′,N′,N″-Pentamethyldiethylenetriamine (PMDETA) and CuBr were supplied by Sigma-Aldrich (USA). Additional chemical reagents were acquired from Beijing Chemical Works (China).

The *A. colemani* and *M. persicae* were obtained from Tuidongzhe Biotechnology Co. (China). All insects were maintained in an artificial climate chamber under controlled conditions with a temperature of (24±2)°C, relative humidity of (70±5)%, and a 14 h:10 h light:dark photoperiod. The *M. persicae* were reared on tobacco seedlings. *Aphidius colemani* were propagated using *M. persicae*, while adult parasitic wasps were provided with a 15% sucrose solution as a dietary source.

2.2. SPc synthesis and clothianidin/SPc complex preparation

The SPc was synthesized following a two-step reaction

process as described by Li *et al.* (2019). In the first step, 2-bromo-2-methylpropionyl bromide (253 mg) was gradually added to a solution of pentaerythritol (25 mg) dissolved in anhydrous tetrahydrofuran (20 mL) containing triethylamine (111.3 mg) at 0°C. The reaction mixture was stirred for 24 h, and the reaction was quenched by the addition of methanol. The resulting product was purified *via* recrystallization in cold ether, yielding star initiator Pt-Br (50 mg, 40% yield). In the second step, Pt-Br was converted into SPc. A reaction flask equipped with a magnetic stirrer was charged with Pt-Br (40 mg), DMAEMA (2.2 g) and anhydrous tetrahydrofuran (8 mL). The mixture was degassed under a nitrogen atmosphere for 30 min to ensure an inert reaction environment. Subsequently, CuBr (46 mg) and PMDETA (110 mg) were added to initiate polymerization at 60°C for 7 h. The reaction was terminated by cooling the mixture to room temperature and exposing it to air. Finally, SPc was obtained as a white powder through freeze-drying. For preparing the clothianidin/SPc complex, clothianidin and SPc solutions were incubated at room temperature for 15 min, and the complex was employed in subsequent experiments.

2.3. Bioactivity assessment of SPc toward parasitic wasps

To assess the potential adverse effects of SPc on *A. colemani*, bioassays were conducted using an SPc aqueous solution. For the eclosion assay, parasitized aphid mummies within 24 h were fully submerged in SPc solutions (50, 30 and 20 mg mL^{-1}) for 30 s. Double-distilled water (ddH_2O) served as the control. The eclosion rate of *A. colemani* was recorded at 24 h intervals. Each treatment consisted of 20 mummies and was replicated three times.

For contact toxicity assessment, the inner walls of pipette tips were coated with SPc solutions (30, 20, 10, 5 and 2 mg mL^{-1}). After natural drying, newly emerged *A. colemani* were introduced into the treated pipette tips. For stomach toxicity assessment, newly emerged *A. colemani* were fed using sterile cotton balls which were fully soaked in 15% sucrose solutions containing SPc (10, 5, 3, 1 or 0.5 mg mL^{-1}). As above, ddH_2O was used as the control. Adults that exhibited no movement when gently touched with a brush were considered dead, and morality was recorded at 2 d post-treatment. Each treatment consisted of 20 parasitic wasps and was repeated three times. The mortality data were analyzed using POLOPlus 2.0 (LeOra Software, USA) to calculate the median lethal concentration (LC_{50}).

2.4. RNA-seq analysis of the SPc-induced lethal mechanism in parasitic wasps

The lethal mechanism of SPc in *A. colemani* at an extremely high concentration was explored using RNA-seq analysis. The *A. colemani* were fed using sterile cotton balls soaked in 0.84 mg mL^{-1} SPc (LC_{50}) or ddH_2O (control) as above. All surviving individuals (n=50) were homogenized in liquid nitrogen at 2 d after immersion, and total RNA was extracted using the RNA Simple Total RNA Kit (Tiangen Biotechnology, China). Three biological replicates were prepared for each treatment.

Transcriptomic libraries were constructed and sequenced using the Illumina HiSeq platform. Raw reads were processed by removing adapter-containing reads, reads with more than 10% ambiguous bases (N), and reads in which over 50% of the bases had a quality score of Q≤10. The high-quality clean reads were then assembled *de novo* using the Trinity to generate a reference transcriptome. The clean reads from each sample were mapped to this reference for downstream analysis. Gene and transcript expression levels were quantified using RNA-seq by expectation maximization (RSEM), and unigene sequences were annotated against the Gene Ontology (GO) database *via* the basic local alignment search tool for translated nucleotide query against protein database (BLASTX). Transcript expression levels are represented as the fragments per kilobase of transcript per million mapped reads (FPKM). Differentially expressed genes (DEGs) were identified using DESeq2, with significance thresholds set at a fold change (FC) of ≥1.5 and a false discovery rate (FDR) of <0.01 (Love *et al.* 2014).

Quantitative real-time PCR (qRT-PCR) was performed to validate the expression levels of selected target genes. The primer sequences are provided in Appendix A. The qRT-PCR reactions were conducted on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA) using the Power SYBR™ Green Master Mix (Applied Biosystems). Each reaction was performed in triplicate under the following thermal cycling conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 30 s. The relative expression levels of target genes were normalized to the housekeeping gene *Large ribosomal subunit protein uL12* (*RPL12*, GenBank: EU190396.1) using the $2^{-\Delta\Delta CT}$ method (Pan *et al.* 2022).

2.5. Measuring the loading capacity of SPc with clothianidin

Pure clothianidin (100 mg) and SPc (100 mg) were mixed in an aqueous methanol solution (10%), and the mixture was dialyzed for 24 h using a regenerated cellulose membrane with a molecular weight cutoff of 2,000 Da (Shanghai Yuanye Biotechnology Co., China). The external dialysis solution was replaced every 6 h with 10% aqueous methanol solution. The dialysis solution was then freeze-dried using a freeze dryer (Beijing Songyuanhuaxing Technology Development Co., China) and weighed. The pesticide loading capacity (PLC) was calculated using the following formula:

PLC (%)=(Mass of clothianidin loaded in complex/Complex mass)×100%

2.6. Analyzing the self-assembly mechanism of the clothianidin/SPc complex

To elucidate the interaction between SPc and clothianidin, the self-assembly mechanism of the clothianidin/SPc complex was investigated by ITC. A 2 mL SPc solution (0.05 mmol L^{-1}) was titrated with 250 μL clothianidin solution (0.5 mmol L^{-1}) using the Nano ITC system (TA Instruments Waters, USA). The calorimetric data from each injection were integrated and analyzed using NanoAnalyze™ software (TA Instruments, USA) to determine

the heat changes associated with their interaction. The Gibbs free energy change (ΔG) was calculated using the following equation:

$$\Delta G=\Delta H-T\Delta S$$

2.7. Particle size measurement and morphological characterization of the clothianidin/SPc complex

The particle sizes of clothianidin (1 mg mL^{-1}) and the clothianidin/SPc complex (1 mg mL^{-1}, mass ratio of 1:1) were measured using the Zetasizer Nano ZS (Malvern Instruments, UK) at 25°C, with three replicates for each solution. The morphological characterization of each sample was conducted by SEM (Regulus 8100, Japan). For SEM sample preparation, a droplet of each sample was deposited onto a silicon wafer and allowed to dry naturally at 25°C. Prior to imaging, the dried samples were coated with a thin layer of gold to enhance the conductivity.

2.8. Contact and stomach toxicity assays of the clothianidin/SPc complex against green peach aphids

The clothianidin/SPc complex was prepared by uniformly incubating clothianidin and SPc at a mass ratio of 1:1. For the contact toxicity assay, 2nd instar nymphs were fully immersed in solutions of clothianidin/SPc complex and clothianidin (clothianidin: 0.2, 0.1 and 0.05 mg L^{-1}) for 20 s, and then transferred onto tobacco seedlings. For stomach toxicity assays, the roots of tobacco seedlings were immersed in the solutions of clothianidin/SPc complex and clothianidin (clothianidin: 0.2, 0.1 and 0.05 mg L^{-1}) for 24 h, and 2nd instar nymphs were then introduced onto the treated seedlings. As above, ddH_2O was used as the control. Mortality was recorded at 2 d after treatment. Each treatment consisted of 30 nymphs, and was repeated three times.

2.9. Safety evaluation of the clothianidin/SPc complex toward parasitic wasps

Three bioassays were used to assess the safety of the clothianidin/SPc complex: eclosion rate of parasitized aphid mummies, contact toxicity, and stomach toxicity. For the eclosion assay, mummies were immersed in solutions of clothianidin/SPc complex (clothianidin: 0.2, 0.1 and 0.05 mg L^{-1}) for 30 s. The number of emerged *A. colemani* was recorded every 24 h, and the eclosion rate was calculated at 7 d. For the contact toxicity assay, the inner walls of glass tubes were coated with solutions of clothianidin/SPc complex and clothianidin (clothianidin: 0.2, 0.1 and 0.05 mg L^{-1}). The coated tubes were allowed to dry naturally before newly emerged *A. colemani* (≤24 h post-emergence) were introduced. For the stomach toxicity assay, newly emerged *A. colemani* were fed using sterile cotton balls that were fully soaked in sucrose solutions (15%) containing either clothianidin/SPc complex (clothianidin: 0.2, 0.1 and 0.05 mg L^{-1}) or clothianidin. As above, ddH_2O was used as the control. The mortality was recorded at 2 d after treatment. Each treatment included 20 mummies or parasitic wasps, and was repeated three times.

To evaluate the effect on the parasitic ability of *A. colemani*, glass tubes were coated with a solution of clothianidin/SPc complex (clothianidin: 0.1 mg L^{-1}), and newly emerged *A. colemani* were then introduced. After 1 h of exposure, the parasitic wasps were transferred onto tobacco seedlings infested with 2nd instar nymphs of *M. persicae*. Following 48 h of parasitism, the *A. colemani* were removed, and the number of mummies was recorded. As above, ddH_2O was used as the control. Each treatment included three parasitic wasps and 30 nymphs, with six replicates.

2.10. Synergistic efficacy assay of the clothianidin/SPc complex and parasitic wasps

To evaluate the synergistic control effect, the clothianidin/SPc complex was co-applied to *M. persicae* with *A. colemani*, and three treatments were evaluated. (1) Biological control by *A. colemani* alone. Newly emerged *A. colemani* were introduced onto tobacco seedlings infested with the 2nd instar aphid nymphs. The *A. colemani* were removed after 48 h, and the parasitism rate was recorded. Each treatment included three parasitic wasps and 30 nymphs. (2) Chemical control by clothianidin/SPc complex alone. The 2nd instar aphid nymphs were immersed in a solution of clothianidin/SPc complex (clothianidin: 0.1 mg L^{-1}) for 20 s, and then transferred onto tobacco seedlings, which had been pre-soaked in the same solution for 24 h. Mortality was recorded at 2 d post-treatment. Each treatment included 30 nymphs. (3) Co-application of *A. colemani* and the clothianidin/SPc complex. Newly emerged *A. colemani* were introduced into glass tubes pre-coated with a solution of clothianidin/SPc complex (clothianidin: 0.1 mg L^{-1}). Meanwhile, nymphs and tobacco seedlings were immersed in the same solution for 20 s and 24 h, respectively. Treated nymphs and *A. colemani* were then introduced onto the seedlings. The *A. colemani* were removed after 48 h, and the mortality was recorded. Each treatment included three parasitic wasps and 30 nymphs. All treatments were repeated four times.

2.11. Data analysis

All statistical analyses were conducted using SPSS 27.0.1 software (SPSS Inc., USA). Descriptive statistics are presented as mean values with standard errors of the mean. One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test was used for multiple comparisons, while the independent *t*-test was applied for pairwise comparisons. The significance level was set at $P<0.05$.

3. Results

3.1. Negligible toxicity of SPc against parasitic wasps

The potential adverse impacts of SPc on *A. colemani* were extensively evaluated in this study. As shown in Fig. 1-A, the SPc exhibited minimal impacts on the eclosion of *A. colemani* from mummies, even at its highest tested concentration (50 mg mL^{-1}) where the eclosion rate remained at 73.3%. As expected, the SPc caused mortality in *A. colemani via* the contact film method, with an LC_{50} of 8.52 mg mL^{-1} (Fig. 1-B; Appendix B). However, *A. colemani* was more sensitive to SPc through oral feeding, with an LC_{50} of 0.84 mg mL^{-1} (Fig. 1-C; Appendix B). Importantly, the application of SPc

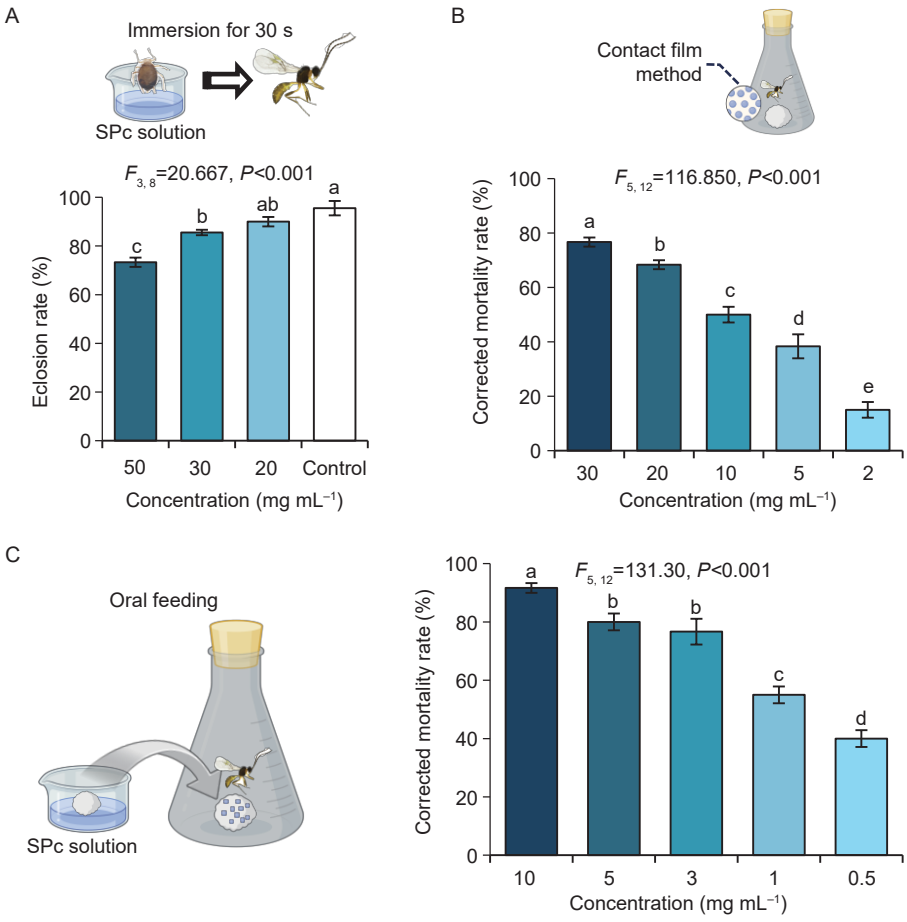


Fig. 1 Biological safety of star polycation (SPc) toward parasitic wasps. A, eclosion rate of *Aphidius colemani* treated with SPc solution, with ddH_2O used as the control. Each treatment consisted of 20 mummies with three replicates. Different letters indicate significant differences according to the Tukey's HSD test (P<0.05). B, contact toxicity of SPc against *A. colemani*. Glass test tubes were coated with SPc solution, and *A. colemani* were then introduced. The corrected mortality was calculated at 2 d after treatment. Each treatment consisted of 20 parasitic wasps with three replicates. C, stomach toxicity of SPc against *A. colemani*. Sterile cotton balls soaked in SPc solution were used to feed the parasitic wasps. The corrected mortality was calculated at 2 d after treatment. Each treatment consisted of 20 parasitic wasps with three replicates.

did not adversely affect the eclosion or survival of *A. colemani* at the working concentration of 0.1 mg mL^{-1}.

3.2. Energy metabolic imbalance in parasitic wasps induced by SPc

Given that *A. colemani* exhibited higher sensitivity to SPc *via* oral feeding, the feeding method was used to investigate the potential mechanism of SPc-induced mortality. RNA-seq analysis was conducted on six high-quality samples, and Pearson correlation tests were conducted (Appendices C and D). Compared to the control group (ddH_2O), 152 genes exhibited significantly differential expression following the SPc treatment, including 100 up-regulated and 52 down-regulated genes (Fig. 2-A). These DEGs were categorized into biological processes, cellular components and molecular functions, which were significantly enriched in several pathways such as translation, cytosolic large ribosomal subunit, structural constituent of ribosome, and others (Fig. 2-B). A notable finding was the significant up-regulation of several ribosomal protein genes (Fig. 2-C). Specifically, *large ribosomal subunit proteins eL* (*RPL*) 27, 29 and 36, as well as *small ribosomal subunit proteins uS* (*RPS*) 11, 16, 21 and 26, were clearly up-regulated after oral feeding with SPc. Finally, qRT-PCR analysis confirmed that the expression patterns of key genes aligned with the transcriptomic data (Fig. 3). For instance, the expression levels of *RPS21*, *RPL29* and *RPS16* increased by 11.4-, 10.9- and 10.4-fold in the parasitic wasps fed with SPc, respectively.

3.3. Self-assembly of SPc with clothianidin *via* hydrogen bonding and Van der Waals forces

The PLC of SPc was determined to be 18.4% toward clothianidin by the freeze-drying method (Appendix E), according to the previous interpretation of ITC data (Ross and Subramanian 1981). The strong binding affinity between SPc and clothianidin, as indicated by a high association constant (Ka, 1.53×10^5 L mol^{-1}) and the negative ΔG (−29.56 kJ mol^{-1}), suggested a spontaneous binding process (Fig. 4). Furthermore, the negative values of enthalpy change (ΔH, −121.9 kJ mol^{-1}) and entropy change (ΔS, −309.7 J mol^{-1} K^{-1}) indicated that hydrogen bonding and Van der Waals forces resulted in the self-assembly of the clothianidin/SPc complex.

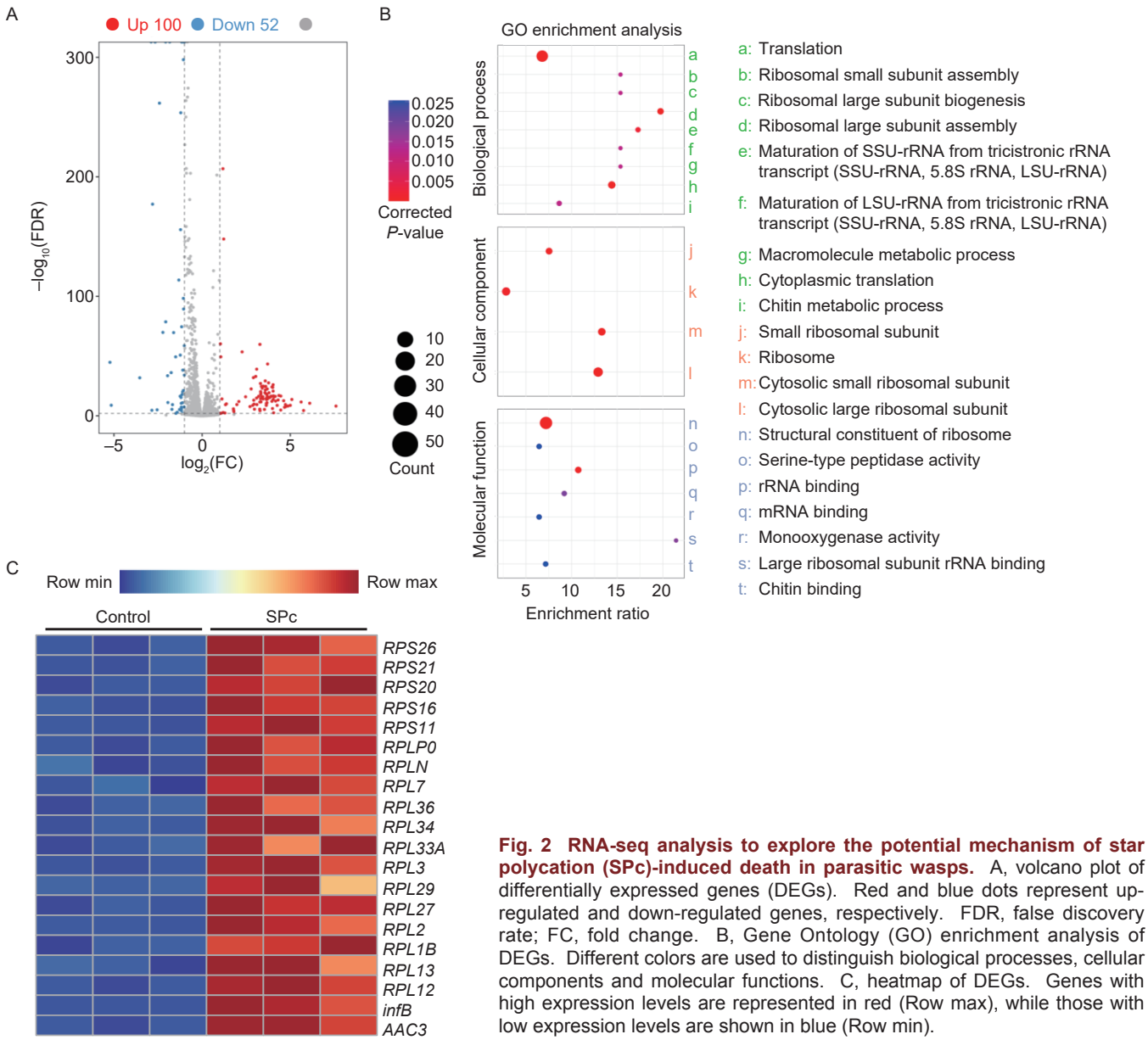


Fig. 2 RNA-seq analysis to explore the potential mechanism of star polycation (SPc)-induced death in parasitic wasps. A, volcano plot of differentially expressed genes (DEGs). Red and blue dots represent up-regulated and down-regulated genes, respectively. FDR, false discovery rate; FC, fold change. B, Gene Ontology (GO) enrichment analysis of DEGs. Different colors are used to distinguish biological processes, cellular components and molecular functions. C, heatmap of DEGs. Genes with high expression levels are represented in red (Row max), while those with low expression levels are shown in blue (Row min).

3.4. Nanometerization of clothianidin by SPc

The DLS and SEM were employed to characterize the clothianidin/SPc complex (Fig. 5; Appendix F). At the mass ratio of 1:1, the particle size of SPc-loaded clothianidin was significantly reduced from 6,554.87 to 467.84 nm. Representative SEM images revealed that the complexation with SPc changed the morphology of clothianidin, facilitating the transformation of irregular large aggregates into nearly spherical nanoparticles.

3.5. Enhanced contact and stomach toxicities of SPc-loaded clothianidin against green peach aphids

For contact toxicity, the enhanced bioactivity of the clothianidin/SPc complex was only observed at a concentration of 0.2 mg L^{-1}, with a 15% increase in mortality (Fig. 6-A). In contrast, the stomach toxicity of the clothianidin/SPc complex was consistently stronger than that of free clothianidin at all tested concentrations, with the corrected

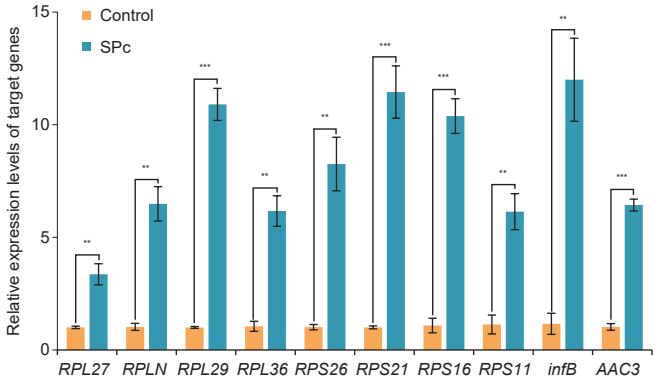


Fig. 3 Validation of differentially expressed genes in parasitic wasps by qRT-PCR. SPc, star polycation. The relative mRNA levels of target genes were normalized to the abundance of the *RPL12* gene. Each treatment included three independent samples. Asterisks indicate significant differences determined by the independent *t*-test (**, $P<0.01$, ***, $P<0.001$).

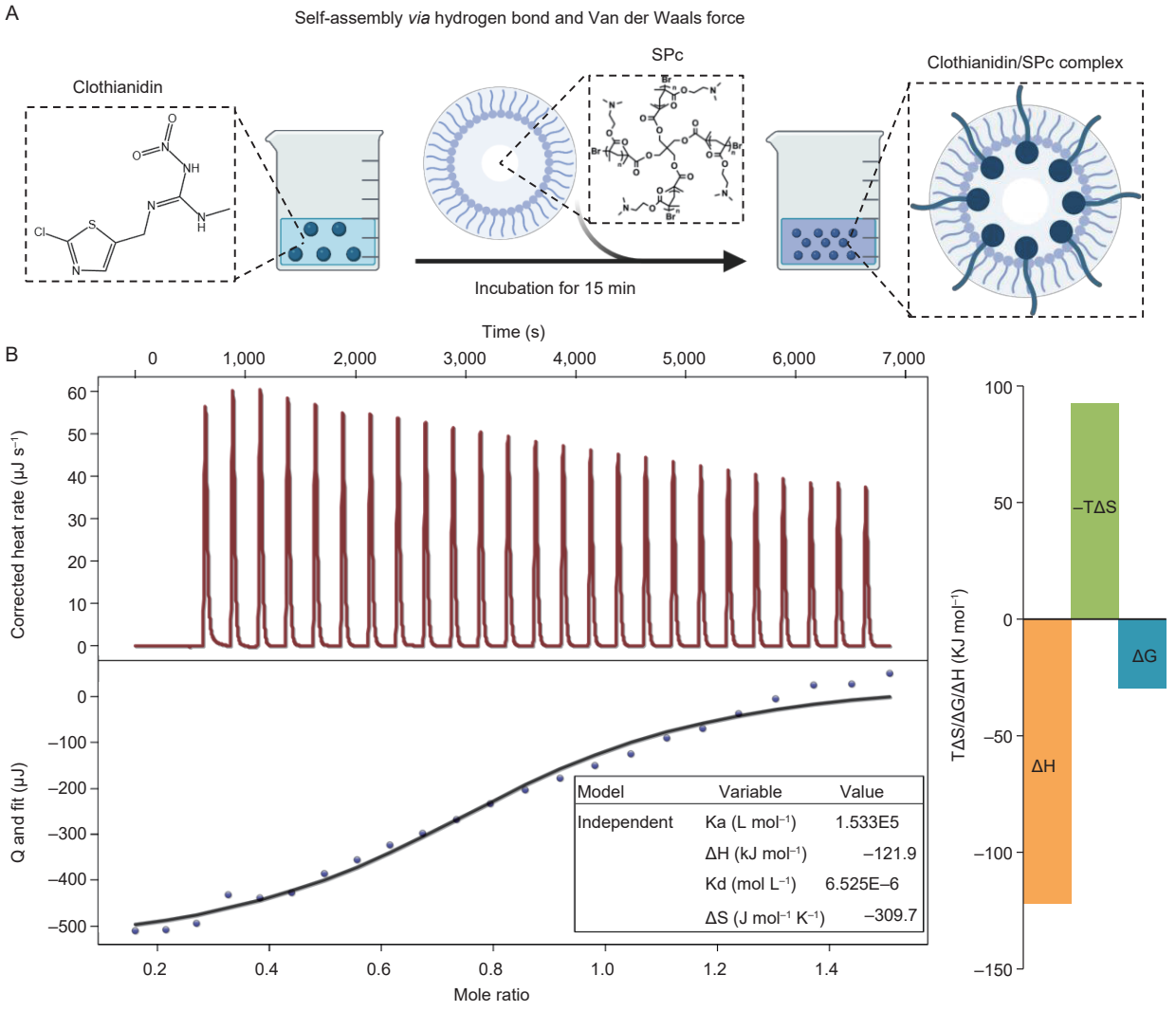


Fig. 4 Self-assembly of the clothianidin/star polycation (SPc) complex. A, schematic diagram of the self-assembly process. B, isothermal titration calorimetry (ITC) titration of clothianidin into the SPc solution. A 2 mL SPc solution (0.05 mmol L^{-1}) was titrated with 250 μL clothianidin solution (0.5 mmol L^{-1}). The experimental temperature was 25°C.

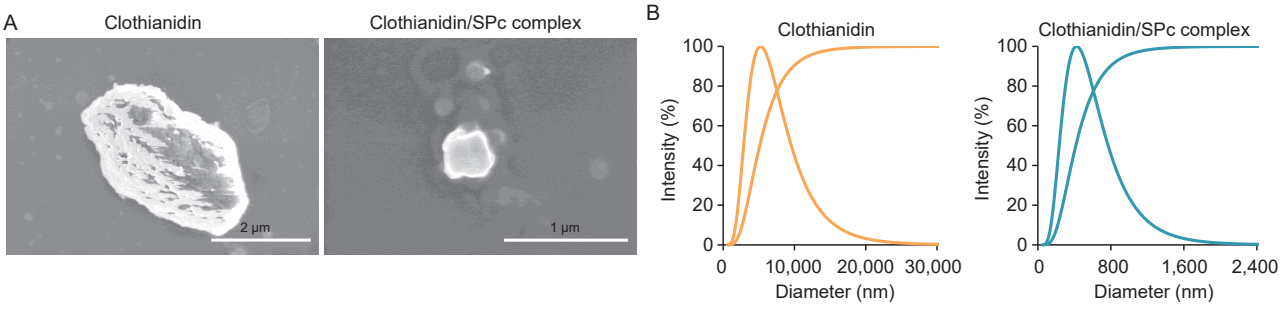


Fig. 5 Morphological characterization and particle size distributions of clothianidin and the clothianidin/star polycation (SPc) complex. A, scanning electron microscopy (SEM) images of clothianidin and the clothianidin/SPc complex. The clothianidin concentration was 1 mg mL^{-1}. B, particle size distributions of the above solutions.

mortalities improved by 28% (0.2 mg L^{-1}), 23% (0.1 mg L^{-1}) and 16% (0.05 mg L^{-1}) at 2 d after oral feeding (Fig. 6-B).

3.6. Relative safety of the clothianidin/SPc complex toward parasitic wasps

As shown in Fig. 7-A, the immersion of parasitized aphid mummies in the clothianidin/SPc complex solution did not affect the normal eclosion of *A. colemani*, and the lowest eclosion rate was 96.7% (10 mg mL^{-1}). At a concentration of 0.1 mg mL^{-1}, the parasitism of *A. colemani* toward *M. persicae* was reduced by only 5% compared to the control (Fig. 7-B). In addition, the dose-dependent experiments showed no

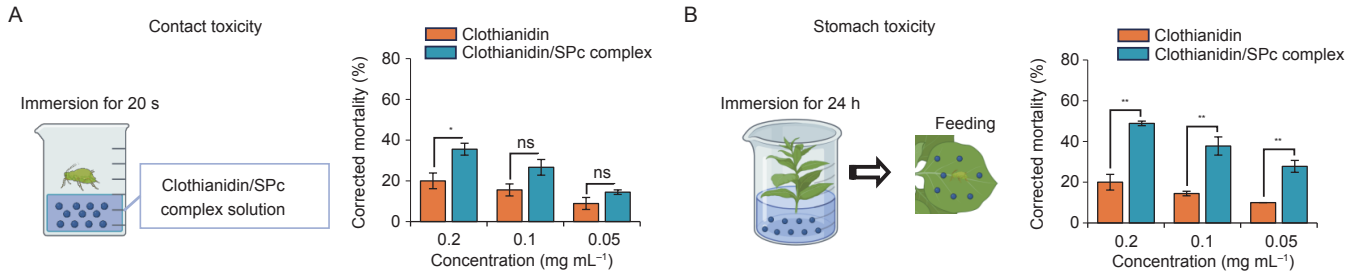


Fig. 6 Contact toxicity (A) and stomach toxicity (B) of the clothianidin/star polycation (SPc) complex against green peach aphids. *Myzus persicae* were directly immersed in a solution of the clothianidin/SPc complex (mass ratio of 1:1) with ddH_2O as the control. The number of dead aphids was recorded at 2 d after treatment. Each treatment included 30 aphids with three replicates. Asterisks indicate significant differences determined by the independent *t*-test (*, $P<0.05$, **, $P<0.01$), and ns indicates no significant difference. Roots of tobacco seedlings were immersed in the above-mentioned solutions for 24 h and then used to feed aphids. The number of dead aphids was recorded at 2 d after treatment. Each treatment included 30 aphids with three replicates.

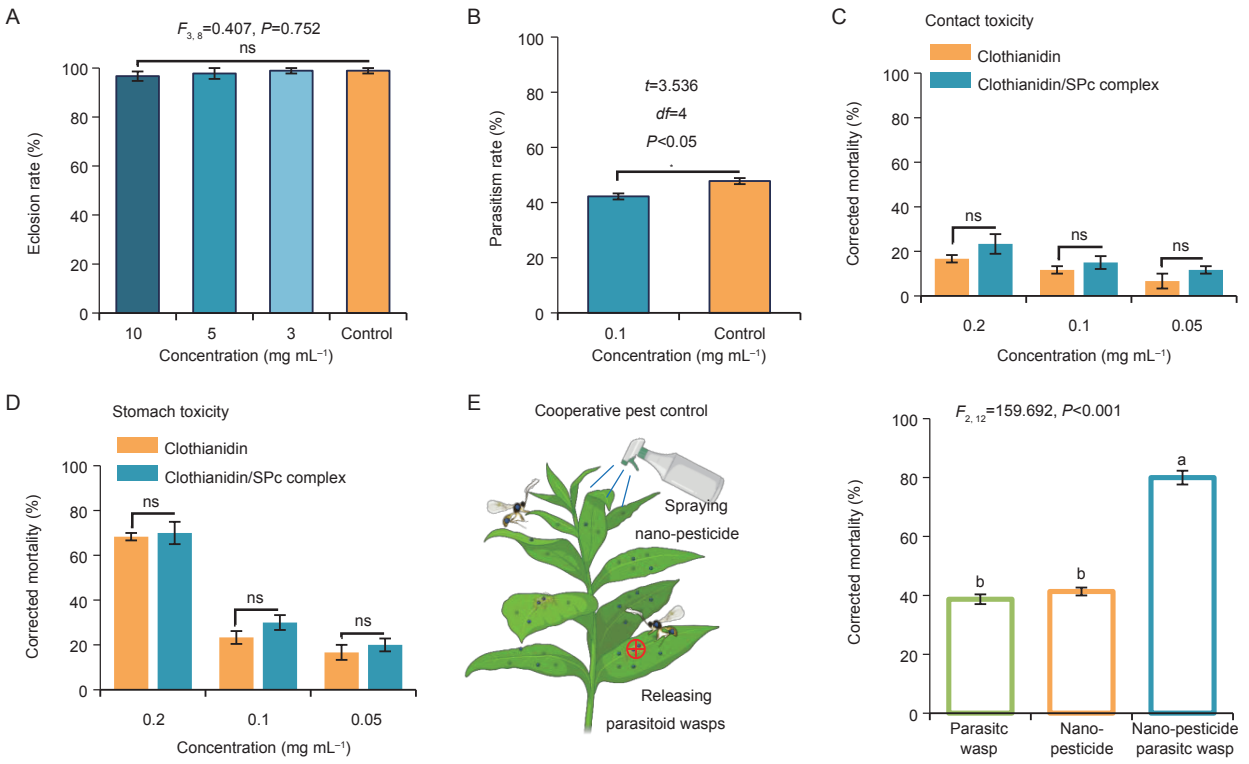


Fig. 7 Safety of the clothianidin/star polycation (SPc) complex and its synergistic application with parasitic wasps. A, eclosion rate of *Aphidius colemani* treated with the clothianidin/SPc complex. Parasitized aphid mummies were immersed in a solution of the clothianidin/SPc complex with ddH_2O as the control. The eclosion rate was calculated at 7 d. Each treatment included 20 mummies with three replicates. ns indicates no significant difference. B, parasitism rate of *A. colemani* treated with the clothianidin/SPc complex. Glass tubes were coated with a solution of the clothianidin/SPc complex, and *A. colemani* were then introduced, transferred after 1 h of exposure, and allowed to parasitize for 48 h. The parasitism rate was assessed at 14 d post-treatment. Each treatment included three parasitic wasps and 30 aphids with six replicates. Asterisks indicate significant differences determined by the independent *t*-test (*, $P<0.05$). C, contact toxicity of the clothianidin/SPc complex against *A. colemani*. Glass tubes were coated with a solution of the clothianidin/SPc complex, and *A. colemani* were then introduced. The mortality was recorded at 2 d after treatment. Each treatment included 20 parasitic wasps with three replicates. D, stomach toxicity of the clothianidin/SPc complex against *A. colemani*. Sterile cotton balls were soaked in the above formulations and used to feed parasitic wasps. The mortality was recorded at 2 d after treatment. Each treatment included 20 parasitic wasps with three replicates. E, cooperative pest control *via* the nano-pesticide and parasitic wasps. The tested clothianidin concentration was 0.1 mg L^{-1}. The mortality was recorded at 2 d after treatment. Each treatment included three parasitic wasps and 30 aphids with four replicates. Different letters indicate significant differences according to the Tukey's HSD test ($P<0.05$).

significant difference in bioactivity between the clothianidin/SPc complex and clothianidin alone toward the adults of parasitic wasps (Fig. 7-C and D). The corrected mortality of *A. colemani* fed with the clothianidin/SPc complex (0.1 mg L^{-1}) was 30%, compared to 23% for those fed with clothianidin alone.

3.7. Synergistic application of the clothianidin/SPc complex with parasitic wasps

Cooperative pest control was successfully achieved through the co-application of the nano-pesticide associated with *A. colemani*. As shown in Fig. 7-E, the application of *A. colemani* alone exhibited a limited control effect, achieving only 38.7% corrected mortality (parasitism rate) in *M. persicae*. Similarly, the corrected mortality reached 41.3% in the aphids treated with only the clothianidin/SPc complex (0.1 mg L^{-1}). However, the cooperative application of the clothianidin/SPc complex associated with *A. colemani* led to the highest corrected mortality (80.0%), demonstrating their synergistic effect on aphids.

4. Discussion

Aphidius colemani is a crucial biological control agent that is widely used in aphid management (Lenteren 1988). Therefore, determining the safe dosage range and elucidating the lethal mechanism of SPc to minimize unintended ecological risks are important. In this study, immersion in an SPc solution showed no significant influence on the eclosion of *A. colemani*. Since *A. colemani* larvae develop inside the host aphid, the mummification process likely provides a protective mechanism that shields the parasitoid larvae from external environmental stressors (Longley 1999). The solid outer shell acts as a natural physical barrier that mitigates the potential negative effects of SPc exposure. In this study, the stomach toxicity of SPc was stronger than its contact toxicity against *A. colemani*. Consistent with previous studies, the toxicity of SPc is highly dependent on the exposure route. For instance, in *Harmonia axyridis*, oral exposure to SPc has an LC_{50} of 19.85 mg mL^{-1}, whereas immersion exposure has a significantly higher LC_{50} of 43.96 mg mL^{-1} (Dong *et al.* 2022). Similarly, mixing graphene oxide (GO) with diet leads to various behavioral and developmental defects in the offspring of fruit flies. However, when fruit flies are exposed to dry carbon nanomaterials, the poor adhesion of these materials allows the flies to remove them through grooming behavior, without affecting mobility or survival (Liu *et al.* 2009; Priyadarsini *et al.* 2019). The differences in biological toxicity between nanocarriers may depend on their biophysical properties, but they generally exhibit dose-dependent effects. Studies have found that titanium dioxide (TiO_2) nanocarriers can induce premature hatching in zebrafish embryos in a dose-dependent manner to cause embryotoxicity (Lu *et al.* 2022). These findings highlight the need to consider toxicity variations arising from different exposure routes and environmental accumulation.

An RNA-seq analysis was conducted to elucidate the lethal mechanism of SPc at an extremely high concentration. Several ribosomal protein genes were up-regulated after oral feeding with SPc. RPL27, RPL29 and RPL36 are components of the large ribosomal subunit, while RPS11, RPS16, RPS21 and RPS26 are components of the small ribosomal subunit (Anger *et al.* 2013; Wang *et al.* 2015). Ribosomes are large ribonucleoprotein complexes that play a central role in protein synthesis, and the small ribosomal subunit is responsible for mRNA recognition and tRNA pairing to ensure accurate translation (Brimacombe 1992; Kramer *et al.* 2009). In addition, *translation initiation factor IF-2* (*infB*), which is essential for initiating protein synthesis and GTP hydrolysis during 70S ribosome assembly, was also up-regulated after oral feeding with SPc (Cenatiempo *et al.* 1987; Milon *et al.* 2010). Another highly expressed gene was *ADP/ATP carrier protein 3* (*AAC3*), which regulates ATP transport between the mitochondrial matrix and cytoplasm to ensure the energy supply (Gnipová *et al.* 2015). Our results suggested that the SPc might induce excessive activation of ribosomal genes, thereby accelerating protein synthesis beyond normal physiological limits. Since protein synthesis is an energy-intensive process, an uncontrolled increase in protein production may lead to a sharp rise in energy demand. If energy replenishment mechanisms fail to compensate for this increased consumption, energy depletion may occur and disrupt physiological homeostasis, potentially leading to cell death. In the short term, this imbalance may manifest as metabolic stress that impairs normal cellular functions (Gomes *et al.* 2016). Our findings are consistent with previous studies showing that SPc exposure induces systemic oxidative stress in *Drosophila melanogaster* (Yan *et al.* 2022). Similarly, extremely high concentrations of SPc have been shown to act as a stressor that promotes energy synthesis in *Arma custos*, which leads to excessive metabolic activity and ultimately results in death (Wu *et al.* 2024). Based on these findings, the lethal mechanism of SPc involves the excessive activation of protein synthesis in *A. colemani*, which disrupts the metabolic balance and triggers endogenous oxidative stress.

The PLC of SPc was calculated to be 18.4% for clothianidin, comparable to other compounds such as sugiyama (17.09%) and camptothecin (16.88%) (Yan *et al.* 2021; Huang *et al.* 2024). Similar loading efficiencies have been reported for other nanomaterials, such as modified mesoporous silica nanoparticles (MSN) which exhibit a loading rate of 12.73% for diquat dibromide (Shan *et al.* 2019). In this study, the SPc could assemble with clothianidin into the clothianidin/SPc complex *via* hydrogen bonding and Van der Waals forces. Hydrogen bonds are a specific type of dipole-dipole interaction between hydrogen donors and acceptors, while Van der Waals forces are common non-covalent interactions that contribute to structural assembly (Luo *et al.* 2015). Similar binding mechanisms have been observed with other nanomaterials. For instance, carbon black nanoparticles interact with tebuthiuron *via* hydrogen bonding and Van der Waals forces, while gold nanoparticles form stable multilayer ultrathin films with polymers through hydrogen bonding (Jiang *et al.* 2008; Prete *et al.* 2017). Depending on the chemical structures and functional groups of exogenous compounds, the SPc can also be engaged in hydrophobic associations and electrostatic interactions (Yan *et al.* 2019; Jiang *et al.* 2024). The dominant interaction forces are usually dependent on the chemical structures, and the diversity of functional groups in SPc is conducive to expanding the field of SPc applications. Our results revealed that complexation with SPc reduced the particle size of clothianidin down to the nanoscale. This size reduction aligns with previous findings that the SPc can encapsulate various

pesticide molecules within its cavities, and achieve pesticide nanometerization. With the help of SPc, the particle sizes of osthole and thiocyclam were reduced to 17.66 and 52.74 nm, respectively (Yan *et al.* 2021; Wang *et al.* 2023). Notably, the small sizes of nano-pesticides have been shown to prolong insecticidal activity for sustainable pest control (Gao *et al.* 2025).

In this study, the clothianidin/SPc complex displayed stronger stomach toxicity against *M. persicae* than clothianidin alone. A previous study suggested that the SPc can increase the effective contact area and adhesion performance of pesticides to target organisms, while it further facilitates pesticide uptake to improve bioactivity (Jiang *et al.* 2023). Similarly, synthetic nano-chitin whiskers loaded with imidacloprid, thiamethoxam and oxydemeton-methyl exhibit significantly higher bioactivity against wheat aphids due to their small particle sizes and large contact areas (Li *et al.* 2021). As a neurotoxic insecticide, clothianidin exhibits strong selectivity against hemipteran and hymenopteran pests, and this specificity was not altered by SPc (Liu *et al.* 2014). Previous studies have shown that although SPc enhances the control efficacy of tetraniliprole against *S. litura*, the prepared tetraniliprole/SPc complex shows no notable lethal effects on predatory stink bugs (Wu *et al.* 2024). Similarly, a water-dispersible colloidal formulation of cypermethrin shows high bioactivity against mosquitoes at low concentrations, while exerting minimal adverse effects on non-targets (Balaji *et al.* 2017). Moreover, silica nanoparticles sprayed on plants can reduce pest populations, while simultaneously enhancing the attraction of natural enemies through plant volatiles (Thabet *et al.* 2021). This study provides a promising approach for integrating nano-pesticides with biological control strategies to promote sustainable pest management.

As expected, the synergistic use of the clothianidin/SPc complex associated with *A. colemani* significantly enhanced the control efficacy against green peach aphids. Previous studies have shown that integrating SPc-loaded pesticides with biological control agents can lead to highly effective pest management outcomes (Dong *et al.* 2022; Wu *et al.* 2024). Similarly, researchers have developed nano-pesticides by loading chlorfenapyr with chitosan, which can not only improve the control efficacy against *Spodoptera frugiperda* but also minimizes its toxicity to honeybees (Hou *et al.* 2023). The synergy based on nano-pesticides and natural enemies establishes a "rapid population reduction — sustainable control" strategy by leveraging both functional enhancement and ecological compatibility. For field applications, we recommended an integrated pest management approach of first applying a low dose of nano-pesticides to reduce the population density of target pests, followed by the release of natural enemies. Alternatively, nano-pesticides can be applied during the egg or pupal stages of natural enemies to maximize their survival and efficacy. This strategy optimizes the high targeting efficiency of nano-pesticides while minimizing adverse effects on natural enemies, which is beneficial for pest control practitioners and environmental regulatory agencies, making it a promising solution for sustainable pest control.

5. Conclusion

In summary, this study successfully developed an efficient clothianidin nano-pesticide and explored its synergistic application with *A. colemani*, which successfully achieved perfect cooperative pest control while reducing pesticide application and minimizing the risks to non-targets. Initially, we demonstrated the negligible toxicity of SPc, and its application did not adversely affect the emergence or survival of *A. colemani* at the working concentration. The oral feeding of SPc at an extremely high concentration led to the up-regulation of ribosomal protein genes and energy metabolism-related genes, which disrupted the metabolic homeostasis and induced endogenous oxidative stress. Subsequently, the SPc was shown to self-assemble with clothianidin *via* hydrogen bonding and Van der Waals forces, and the complexation with SPc could reduce the particle size of clothianidin down to the nanoscale. As expected, the clothianidin/SPc complex displayed stronger bioactivity than free clothianidin against *M. persicae*. Importantly, *A. colemani* exhibited low sensitivity to the clothianidin/SPc complex, with nearly no adverse impacts on their eclosion or parasitism. Furthermore, the co-application of the clothianidin/SPc complex with *A. colemani* displayed an excellent control effect, achieving both rapid pest suppression and sustainable control. This innovative approach ultimately achieved perfect cooperative pest control *via* nano-pesticide and parasitic wasps, which offers a promising strategy for maintaining long-term agricultural ecological balance.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in scientific writing

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Ethical statements

All applicable international, national and institutional guidelines for the care and use of animals were followed.

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