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Preparation of Nanoscale Indoxacarb by using Star Polymer for Efficiency Pest Management

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Abstract: The utilization efficiency of conventional pesticides is relatively low in agricultural production, resulting in excessive application and environmental pollution. The efficient utilization of pesticides is crucial for promoting sustainable agriculture, and the development of nanopesticides presents a promising solution to the challenges associated with traditional pesticides. In order to explore an efficient application method for indendicarb (IDC), a star polymer nanocarrier (SPc) was employed to design and construct an efficient nanodelivery system for IDC. In this study, the morphology and physicochemical properties of the complex were determined, and its bioactivity and control efficacy were assessed using leaf-dipping and field spraying methods. The results show that IDC could be spontaneously incorporated into the hydrophobic core of SPc via hydrophobic association. This assembly disrupted the self-aggregated structure of IDC and significantly reduced its particle size to nanoscale. Furthermore, IDC emulsifiable concentrate (IDC EC) demonstrated improved adhesion to plant leaves with the aid of SPc, increasing retention from 8.083 to 10.418 mg/cm^2. The LC_{50} (1d) of IDC EC against *Plutella xylostella* (Linnaeus) and *Pieris rapae* (Linnaeus) decreased by 6.784 and 1.931 times, respectively, with the addition of SPc. The inclusion of SPc increased the control effect of IDC EC by up to 8.28% (7d, 3000×) for *P. xylostella* and 12.53% (3d, 8000×) for *P. rapae*. This reveals that the IDC EC + SPc formulation exhibits superior insecticidal activity against these two highly destructive insect pests. This study successfully developed a novel nanodelivery system for the efficient application of IDC, which has the potential to reduce overapplication and promote sustainable agricultural practices.

Keywords: indoxacarb; nanodelivery system; star polymer; *Plutella xylostella*; *Pieris rapae*; improved insecticidal efficacy; sustainable agriculture

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1. Introduction

Pesticides serve as vital inputs for agricultural production, safeguarding approximately one-third of all agricultural products globally. They have played an irreplaceable role in promoting food production and ensuring food security [1–3]. Over the past decade, global pesticide application has grown by 20% by volume [4]. However, most traditional synthetic pesticides present a series of shortcomings, such as coarse particles, instability, and poor dispersibility, which result in low bioactivity and availability [2,5]. It has been estimated that only 1–25% of applied pesticides actually reach the target organisms, with the remainder being released into the environment as a potential hazard [6–9]. Furthermore, apart from environmental contamination, the overuse or misuse of chemical pesticides can contribute to the development of insect resistance and pose harm to non-target

organisms [1,2,6]. Hence, there is an urgent need for advanced technologies to address these challenges and facilitate the sustainable development of agriculture.

As an emerging and promising research field, nanotechnology offers innovative methods for designing and delivering active ingredients (AIs) at the nanoscale [10–14]. Nanopesticides are anticipated to address the primary limitations of synthetic pesticides by reducing runoff, increasing plant uptake, improving foliar adhesion, and enhancing bioavailability [6,14]. Nanopesticides can be prepared via two pathways: direct processing into nanoparticles or loading pesticides in nanocarriers [6,8,15]. In particular, polymeric nanomaterials have been extensively employed to construct nanoscale delivery platforms for AIs, with the aim of reducing the application of AIs and improving their bioactivity [16,17]. Our group has designed and synthesized a star polymer (SPc) that is capable of acting as a nanocarrier to deliver both pesticides and double-stranded RNA (dsRNA) [18,19]. With the assistance of SPc, the plant uptake of pesticides significantly improves, while pesticide residues simultaneously decrease [20,21]. The SPc-loaded pesticides exhibited enhanced selectivity toward natural predators, and the co-application of nanopesticides and predators achieved perfect co-operative pest management [22,23]. Moreover, the SPc-based co-delivery systems for dsRNA and pesticides have been successfully implemented for plant protection [22]. Currently, SPc-based delivery systems have been adopted for the high-efficiency management of plant diseases, pests, and nematode diseases [24,25]. These diverse applications display the excellent potential of SPc as an effective adjuvant for pesticide design.

As a dioxin insecticide, indoxacarb (IDC) is classified as a sodium channel blocker insecticide (SCBI), which can be metabolized by insect esterases or amidases to an N-decarbomethoxylated metabolite (DCJW). This metabolite leads to the flaccid paralysis and death of insects [26]. Due to its novel acting mechanism, IDC serves as an ideal alternative to organophosphorus and pyrethroid insecticides [27,28]. IDC exhibits organism-selective activity, being highly effective against lepidopteran pests while remaining safe for non-target organisms [29,30]. However, its extensive use has raised environmental and ecological concerns. The residues of IDC have been detected in crops, soil, and water [31–34], and it has shown toxicity to aquatic organisms and beneficial insects [29,35–38]. Moreover, the over-reliance on insecticides has resulted in the emergence of widespread insecticide resistance. In order to achieve satisfactory control effects, excessive insecticides than recommended doses are usually applied [39]. Thus, several lepidopteran pests have developed high levels of field-evolved IDC resistance [40–43]. In response to this challenge, researchers have developed nanoparticles to enhance the insecticidal activity of IDC, such as fluorescent mesoporous silica nanoparticles (FL-SiO2 NPs), a β-cyclodextrin-functionalized metal-organic framework (ZIF-90-CD), and carboxylated β-cyclodextrin anchored hollow mesoporous silica (HMS-CD) [44–46]. Whether SPc can optimize the physicochemical properties of IDC to improve its bioavailability and reduce its usage, thereby lessening environmental impact, is an interesting topic.

In this context, we developed an efficient nanodelivery system for IDC to reduce its particle size and enhance its retention. In order to elucidate the self-assembly mechanism of the IDC/SPc complex, we measured the pesticide loading content of SPc toward pure IDC and analyzed their interaction force for self-assembly. Subsequently, we determined the particle size of the IDC/SPc complex and examined its morphology. Additionally, we evaluated the retention of SPc-loaded IDC emulsifiable concentrate (IDC EC) on cabbage leaves. Finally, we demonstrated the enhanced bioactivity and better control efficacy of the IDC EC + SPc formulation against two insect species, *Plutella xylostella* (Linnaeus) and *Pieris rapae* (Linnaeus), in both the laboratory and the field. Our study designed and constructed a novel nanodelivery system to improve the bioactivity of IDC and broaden its application, offering a universal method for the design and development of efficient pesticides.

2. Materials and Methods

2.1. Chemical Reagents

Triethylamine, 2-bromo-2-methylpropionyl bromide, CuBr, N,N,N′,N′,N″-Pentamethyl diethylenetriamine (PMDETA), and 2-(dimethyl-amino) ethyl methacrylate (DMAEMA) were used for the synthesis of SPc. Triethylamine and 2-bromo-2-methylpropionyl bromide were purchased from Heowns BioChem Technologies (Tianjin, China). CuBr (99.999%) and PMDETA (98%) were bought from Sigma-Aldrich (Saint Louis, MO, USA), and DMAEMA (99%) was obtained from Energy Chemical (Shanghai, China). Pure IDC (≥94%) for characterization assays and IDC emulsifiable concentrate (IDC EC, effective content: 150 g/L) for bioactivity assays were acquired from Jiangsu Longdeng Chemical Co., Ltd. (Kunshan, China) and DuPont Co. (Wilmington, DE, USA), respectively. Solvents, such as ethanol and acetone, were purchased from Beijing Chemical Works (Beijing, China).

2.2. SPc Synthesis

The synthesis of SPc was carried out via two steps, following the procedure described by Li et al. [19]. Initially, at 0 °C, 2-bromo-2-methylpropionyl bromide (253 mg, 1.11 mmol) was added dropwise into the solution of pentaerythritol (25 mg, 0.18 mmol) in dry tetrahydrofuran (THF, 20 mL) and triethylamine (TEA, 111.3 mg, 1.11 mmol). After stirring for 24 h at room temperature, methanol was used to quench the reaction. The solvent was then removed under reduced pressure, and the residue was recrystallized in cold ether, obtaining Pt-Br (50 mg, 40%) as a white powder. Subsequently, the Pt-Br initiator (40 mg, 0.055 mmol), DMAEMA (2.2 g, 7.7 mmol) and dry THF (8 mL) were mixed in a flask equipped with a magnetic stirrer and degassed under a nitrogen atmosphere for 30 min. CuBr (46 mg, 0.22 mmol) and PMDETA (110 mg, 0.44 mmol) were then added, and the flask was sealed for polymerization in an oil bath at 60 °C for 7 h. The reaction was quenched by cooling and exposing to air. Following the removal of THF using a rotary evaporator, the crude polymer underwent purification via dialysis in water four times. The purified polymer was then dried under reduced pressure, yielding SPc as a white powder.

2.3. Loading Capacity Measurement

The loading capacity of SPc toward IDC was measured using the freeze-drying method [24]. IDC (70 mg) was dispersed in 10 mL of acetone and mixed with 1 mL of 62.7 mg/mL SPc aqueous solution. The mixture was thoroughly stirred before being diluted to 20 mL with double-distilled water (ddH_2O). The IDC was incubated with SPc for 15 min and then dialyzed for 24 h using regenerated cellulose membranes with a molecular weight cut-off of 2000 Da (Shanghai Yuanye Bio-Technology Co., Shanghai, China) to remove excess IDC. The dialysis buffer (30% acetone aqueous solution) was replaced every 6 h. After dialysis, the solution was freeze-dried using a vacuum freeze-dryer (Beijing Songyuanhuaxing Technology Development Co., Beijing, China) and weighed. This procedure was performed in triplicate. The pesticide loading content (PLC) was calculated using the following equation:

$$\text{PCL (\%)} = \frac{100 \times (\text{weight of IDC loaded in complex})}{\text{weight of IDC-loaded complex}} \quad (1)$$

2.4. Isothermal Titration Calorimetry (ITC) Assay

In order to determine the interaction between SPc and IDC, 1 mL of IDC (0.138 mmol/L) in 5% acetone aqueous solution was titrated with 250 μL of SPc aqueous solution (1 mmol/L) using a Nano ITC (TA Instruments Waters, New Castle, DE, USA). Prior to the assay, the calorimeter cell, reference cell, and syringe were washed with ddH_2O three times. The assay was performed at 25 °C, with an injection size of 10 μL, and was repeated

25 times with an interval of 250 s between each injection. The heat of the interaction for each injection was calculated by integrating the titration peak using the NanoAnalyze software v3.12.0 (TA Instruments Waters, New Castle, DE, USA) [47,48]. The test was conducted at 25 °C, and the change in Gibbs free energy (ΔG) was calculated using the following formula:

$$\Delta G = \Delta H - T\Delta S \quad (2)$$

2.5. Particle Size Measurement and Complex Morphology Characterization

The particle sizes of the IDC/SPc complex at the mass ratio of 1:1 (0.25 mg/mL) and IDC (0.25 mg/mL) in 5% acetone aqueous solution were measured using the Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK) at 25 °C. Each sample was measured three times. The morphologies of the above samples were further observed using a scanning electron microscope (SEM, Regulus 8100, Hitachi, Ltd., Tokyo, Japan). Before observation, 20 μL of each sample was dropped onto the silicon slice to air-dry for 24 h and then coated with platinum material.

2.6. Retention Assay

The 150 g/L IDC EC was mixed with SPc at the mass ratio of 1:1 and then diluted 3000 times to prepare the IDC EC + SPc formulation (0.05 mg/mL). Cabbage (*Brassica oleracea* var. *capitata* Linnaeus) leaves with consistent growth conditions were cut into 7 cm × 7 cm sections along the midvein. These sections were weighed using an electronic balance and then completely immersed in 200 mL of either 0.05 mg/mL IDC EC or IDC EC + SPc formulation for 10 s. The treated sections were weighed after removal until no droplets dropped. Each treatment included 30 replicates. The retention was calculated using the following formula:

$$\text{Retention (mg/cm}^2\text{)} = \frac{\text{blade weight after immersion} - \text{blade weight before immersion}}{\text{superficial area}} \quad (3)$$

2.7. Bioactivity Assay via Oral Feeding in Laboratory

As highly destructive insect pests, *P. xylostella* and *P. rapae* cause significant economic losses for Brassicaceae vegetables. *Plutella xylostella*, in particular, has become one of the most challenging insect pests to control globally, exhibiting resistance to as many as 101 AIs [49,50]. In this context, the toxicities of the IDC EC and IDC EC + SPc formulations were assessed against these two pest species. *Plutella xylostella* and *P. rapae* were collected from the experimental farm of Yantai Research Institute of Agricultural Sciences (latitude: 37.478561° N, longitude: 121.274066° E) in Fushan District, Yantai City, Shandong Province, China. The insects were fed on cabbages and reared under controlled conditions of 25 ± 1 °C, 75 ± 5% relative humidity and a 14 L:10 D photoperiod.

Following the national standard (Guideline for laboratory bioassay of pesticides Part 14: Leaf-dipping method), laboratory bioassays were conducted using the leaf-dipping method. Fresh cabbage leaves were cut into 9 cm diameter discs, cleaned with sterile water, dried, and then immersed in the IDC EC + SPc formulations (mass ratio: 1:1) at the concentrations of 5, 2.5, 1.25, 0.625, 0.313, and 0.1565 mg/L. After immersing for 10 s, the leaf discs were removed, air-dried, and placed into 9 cm culture dishes before inoculating with third instar larvae of *P. xylostella* or second instar larvae of *P. rapae*. Corresponding concentrations of IDC EC were also tested, with ddH_2O serving as the control. The treated insects were reared under normal conditions, and the number of surviving insects was recorded at 24 h and 48 h after the treatment. The concentration-mortality data were analyzed using SPSS 27.0 (SPSS Inc., New York, NY, USA) [51] to calculate the lethal concentration 50 (LC_{50}) values (Analyze: Probit Analyze; Transform: Log base 10). Each treatment

contained 30 larvae and was repeated three times. The efficiency ratio was calculated as the ratio of IDC EC LC_{50} to IDC EC + SPc formulation LC_{50}.

2.8. Control Efficacy Assay via Spraying in Field

Spraying experiments were conducted against *P. xylostella* and *P. rapae* at different sites of the experimental farm of Yantai Academy of Agricultural Sciences (latitude: 37.478561° N, longitude: 121.274066° E) in Fushan District, Yantai City, Shandong Province, China. The cabbage variety was Improved Zhonggan 11, which was in the heading stage during the experiment period. Fifteen plots were set up for each pest, each covering approximately 30 m^2, with consistent cultivation conditions across all plots. The populations of *P. xylostella* and *P. rapae* were surveyed before the spraying experiments.

The control efficacy assay was conducted to explore the synergism and reduction of SPc towards IDC EC at the conventional dose and half the conventional dose. The 150 g/L IDC EC was mixed with SPc at the mass ratio of 1:1 and then diluted 3000, 4000, 6000, and 8,000 times with water to prepare 3000×, 4000×, 6000×, and 8000× IDC EC + SPc formulations. The 8000× and 4000× IDC EC and IDC EC + SPc formulations were applied to control *P. rapae*. The experiment was conducted from 1 to 8 June 2022 with the formulations sprayed on the afternoon of 1 June. The average temperature during the experiment was 17.3–26.1 °C, with relative humidity ranging from 47–92%. The 6000× and 3000× IDC EC and IDC EC + SPc formulations were applied to control *P. xylostella*. The experiment was conducted from 14 to 21 July 2022, and the formulations were sprayed on the afternoon of 14 July. The average temperature during the test was 23.9–27.8 °C, with relative humidity ranging from 73–94%. Water was applied as the control, and each treatment was repeated three times. The spraying was performed using an electric MATABI Super Green16 sprayer (GOIZPER S. Coop., Antzuola, Spain) with an application amount of 45 mL/m^2. Ten plants from each plot were randomly selected as replicates, and the populations of *P. xylostella* and *P. rapae* were recorded at 3 and 7 days after the treatment. According to the study by Yan [18], the dropping rate of insects (DRI) and control efficacy (CE) were calculated using the following formulas:

$$\text{DRI (\%)} = \frac{100 \times (\text{insect number before pesticide application} - \text{insect number after pesticide application})}{\text{insect number before pesticide application}} \quad (4)$$

$$\text{CE (\%)} = \frac{100 \times (\text{DRI in the treatment plot} - \text{DRI in the control plot})}{1 - \text{DRI in the control plot}} \quad (5)$$

2.9. Data Analysis

A one-way ANOVA (Tukey HSD test) and independent t-tests were adopted to analyze the data using SPSS 27.0 (SPSS Inc., New York, NY, USA) [51] at the significance level of $p = 0.05$. Descriptive statistics were presented as the mean value and standard error of the mean.

3. Results

3.1. SPc Spontaneously Complexed with Indoxacarb

Through two simple synthetic steps, we obtained the SPc as a white powder. The self-assembly of the IDC/SPc complex could be easily achieved by straightforwardly mixing and incubating at room temperature for 15 min. The loading capacity of SPc toward indoxacarb is shown in Table 1. Specifically, 62.7 mg of SPc could assemble with 13.4–14.6 mg of IDC, resulting in an average PLC of 18.18%.

Table 1. Loading capacity of SPc toward IDC using freeze-drying method.

Sample Number	Weight of Applied IDC (mg)	Weight of Applied SPc (mg)	Weight of IDC-Loaded Complex (mg)	Weight of IDC Loaded in Complex (mg)	Drug-Loading Content (%)	Average Drug-Loading Content (%)
1	70.0	62.7	76.1	13.4	17.61	
2	70.0	62.7	77.3	14.6	18.89	18.18 ± 0.38
3	70.0	62.7	76.5	13.8	18.04	

Mean ± SE.

The ITC was used to determine the binding force between SPc and IDC, illustrating their self-assembly mechanism (Figure 1). The values of ΔH and ΔS were found to be 9.348 kJ/mol and 130.8 J/mol·K, respectively, and the ΔG was calculated to be −29.65 kJ/mol. The negative ΔG indicated that the self-assembly was spontaneous. The positive values of ΔH and ΔS suggested that the complexation of SPc with IDC occurred via hydrophobic association, implying that IDC could be assembled into the hydrophobic core of SPc. Additionally, the affinity constant (Ka) was 1.560×10^5 M^{-1}, while the dissociation constant (Kd) was 6.409×10^{-6} M. The high Ka and low Kd values indicated a strong interaction between SPc and IDC.

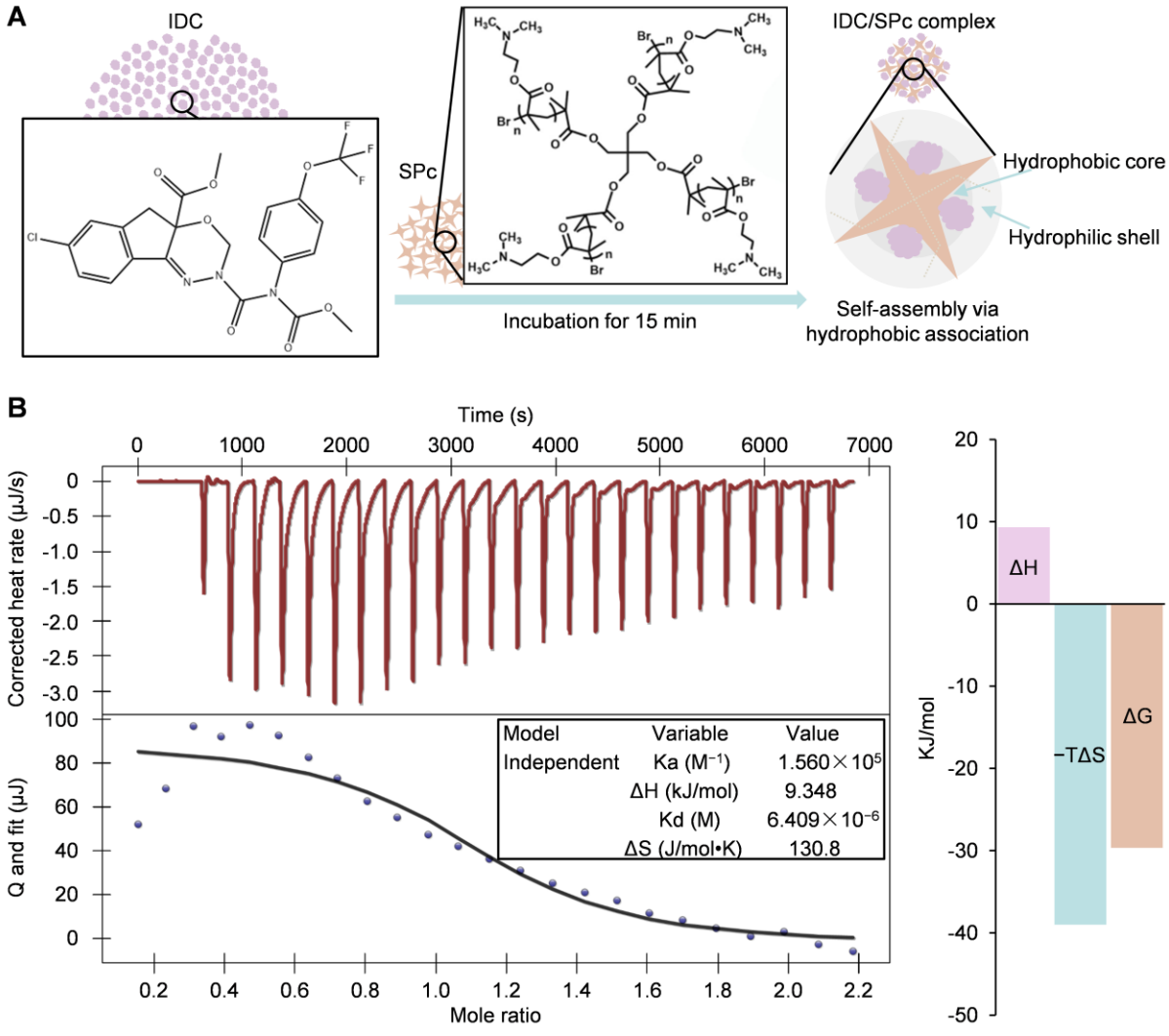

Figure 1. Schematic illustration of IDC/SPc complex (**A**) and ITC assay (**B**). The titration was performed by adding 250 μL of SPc solution (1 mmol/L) into 1 mL solution of IDC (0.138 mmol/L). The test temperature was 25 °C.

3.2. SPc Decreased the Particle Size of Indoxacarb

The particle sizes and polydispersities of the IDC/SPc complex are shown in Table 2 and Figure 2A. The complexation of IDC with SPc at the mass ratio of 1:1 reduced the particle size of IDC from 922.44 to 444.70 nm. A further reduction in the particle size of the IDC/SPc complex was observed at the mass ratio of 1:4.5. Interestingly, the polydispersity of the IDC/SPc complex at the mass ratio of 1:1 significantly decreased to 0.065, while there was no significant reduction at the mass ratio of 1:4.5. The morphological features of IDC and the IDC/SPc complex were examined using SEM (Figure 2B). Representative SEM images further confirmed the above findings, showing that the self-aggregated IDC/SPc complex consisted of smaller particles compared to IDC alone.

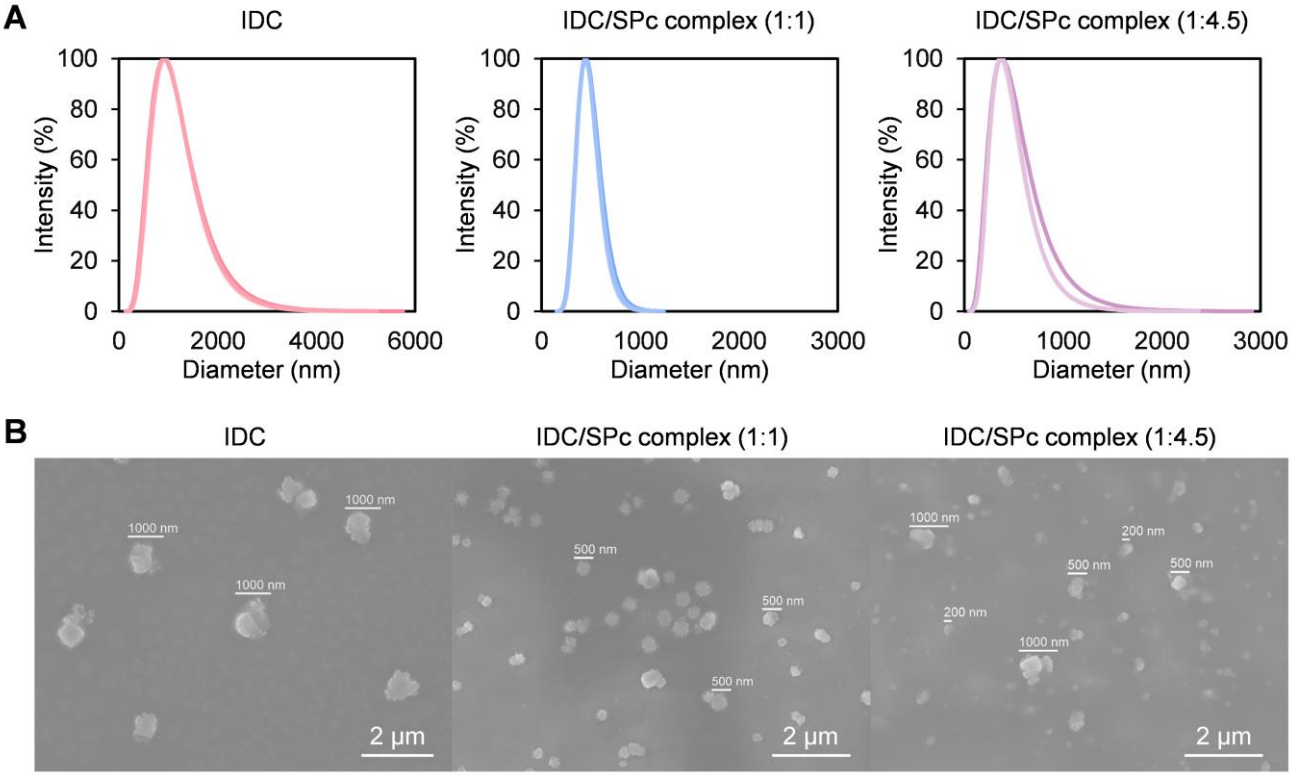

Figure 2. Particle size distributions (**A**) and SEM images (**B**) of IDC and the IDC/SPc complexes at the mass ratios of 1:1 and 1:4.5.

Table 2. Polydispersities and particle sizes of IDC alone and the IDC/SPc complex.

Formulation	Mass Ratio	Sample Number	Polydispersity	Average Polydispersity	Size (nm)	Average Size (nm)
IDC	-	1	0.230	0.211 ± 0.019 a	911.17	922.44 ± 7.41 a
		2	0.210		919.74	
		3	0.193		936.41	
IDC/SPc complex	1:1	1	0.065	0.065 ± 0.006 b	442.3	444.70 ± 1.89 b
		2	0.059		443.36	
		3	0.070		448.43	
	1:4.5	1	0.233	0.247 ± 0.042 a	363.34	373.20 ± 5.18 c
		2	0.214		375.39	
		3	0.294		380.87	
			$F_{2,6}$ =39.584, $p < 0.001$		$F_{2,6}$ = 3136.221, $p < 0.001$	

Means ± SE followed by different letters are significantly different (Tukey HSD test, $p < 0.05$).

3.3. SPc Increased the Retention of Indoxacarb Emulsifiable Concentrate

After immersion in the IDC EC or IDC EC + SPc formulations, the droplets spread and adhered to the leaf surfaces. As shown in Figure 3, the pesticide retention of the IDC EC solution was calculated to be 8.083 mg/cm^2. With the addition of SPc, the pesticide retention increased to 10.418 mg/cm^2, which was 1.3 times higher than that of IDC EC alone.

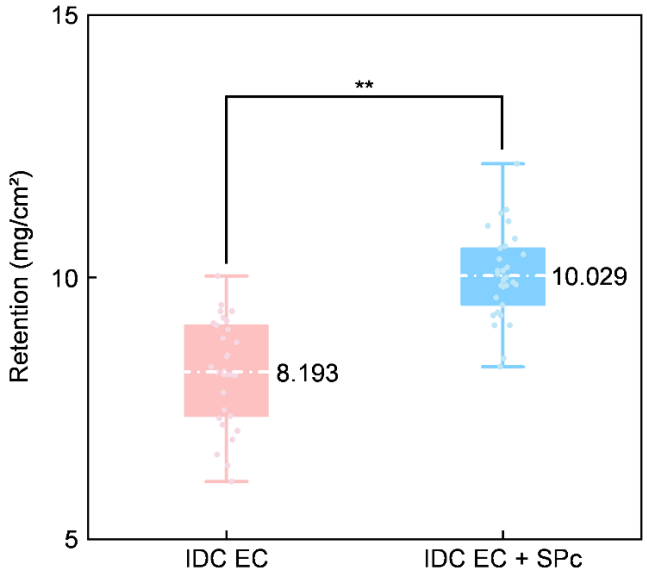

Figure 3. Retention of IDC EC and IDC EC + SPc formulations at the mass ratio of 1:1. Each treatment contained 30 independent samples. The "**" indicates significant differences (independent *t*-test; $p < 0.01$).

3.4. SPc Improved the Bioactivity of Indoxacarb Emulsifiable Concentrate in the Laboratory

The laboratory bioactivities of the IDC EC and IDC EC + SPc formulations were assessed against *P. xylostella* and *P. rapae* using the leaf-dipping method (Figure 4). The results showed that the corrected mortalities of *P. xylostella* and *P. rapae* treated with the IDC EC + SPc formulation were higher at all tested concentrations compared to those treated with IDC EC alone. More specifically, the corrected mortality of *P. xylostella* treated with the IDC EC + SPc formulation significantly increased by 26.09% (1 d) and 24.90% (2 d) at a concentration of 0.313 mg/L. The corrected mortality of *P. rapae* treated with the IDC EC + SPc formulation significantly increased by 21.53% (0.625 mg/L, 1 d), 17.16% (1.25 mg/L, 1 d), 14.34% (1.25 mg/L, 2 d), and 9.40% (2.5 mg/L, 2 d). These findings clearly indicated that the insecticidal activity of IDC EC improved with the aid of SPc.

As shown in Table 3, the LC_{50} value of the IDC EC + SPc formulation against *P. xylostella* decreased to 0.305 mg/L at 1 d after the treatment compared to 2.069 mg/L for IDC EC alone, with a high efficiency ratio of 6.784. Similarly, the LC_{50} value of IDC EC decreased from 1.261 to 0.653 mg/L against *P. rapae* with the aid of SPc, with an efficiency ratio of 1.931. At 2 d after the treatment, the LC_{50} values of the IDC EC + SPc formulation also decreased against the two pest species, with efficiency ratios of 4.261 and 1.583, respectively.

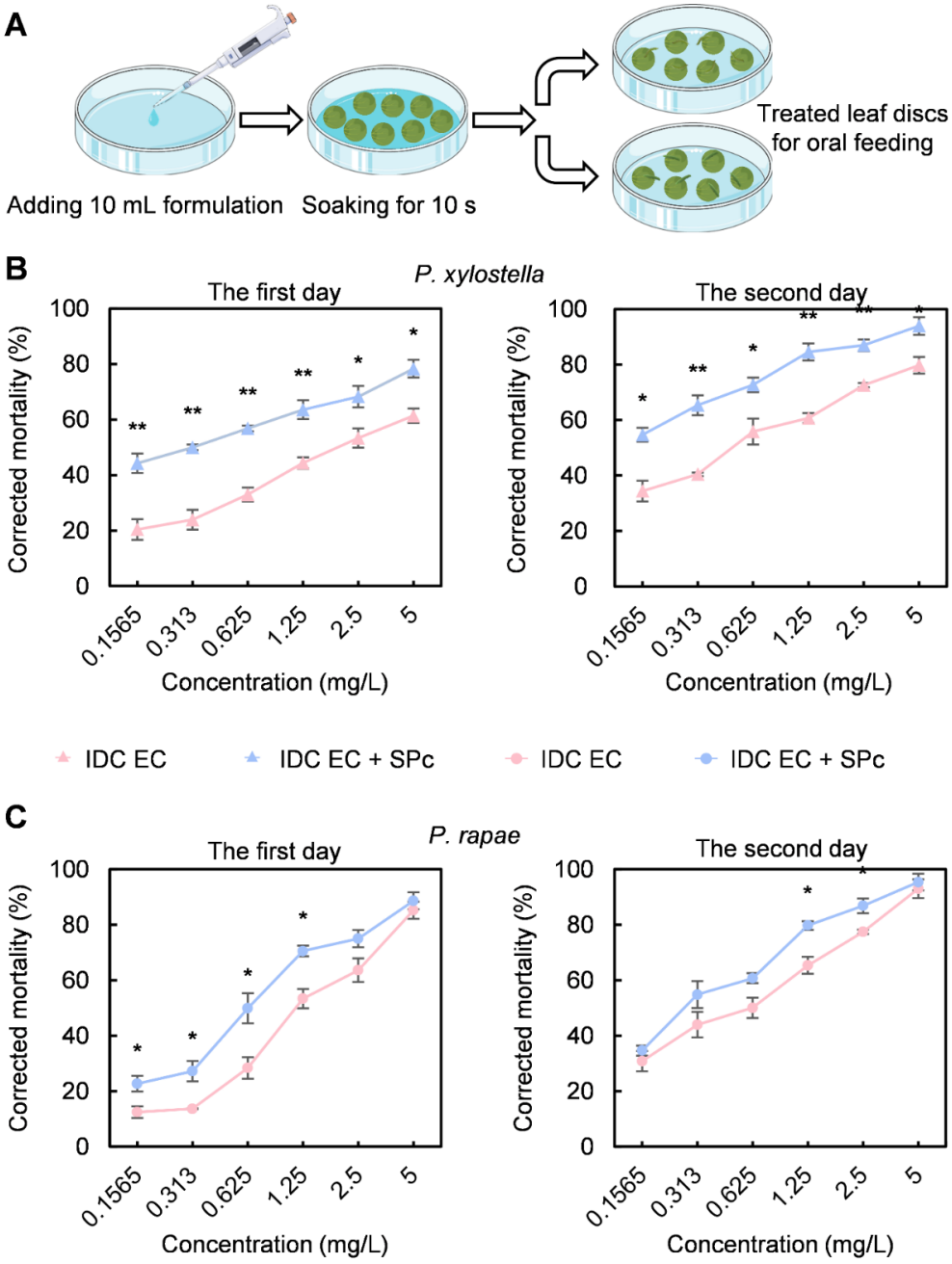

Figure 4. Bioactivity of the IDC EC + SPc formulation against two insect pests. (**A**) Schematic illustration of the leaf-dipping method. (**B**) Bioactivity of the IDC EC + SPc formulation against the third instar larvae of *P. xylostella*. The mortality was recorded at 1 d after the treatment. Each treatment contained 30 larvae and was repeated three times. "*" and "**" indicate significant differences (independent *t*-test; $p < 0.05$ and $p < 0.01$). (**C**) Bioactivity of the IDC EC + SPc formulation against the second instar larvae of *P. rapae*.

Table 3. Toxicity of the IDC EC and IDC EC + SPc formulations against the *Plutella xylostella* and *Pieris rapae*.

Insect Species	Formulation	Day (d)	Toxicity Regression Equation	LC_{50} (mg/L) (95% Confidence Limits)	χ^2 (df)	Efficiency Ratio
P. xylostella	IDC EC	1	y = −0.249 + 0.788x	2.069 (1.158–6.005)	0.148 (4)	6.784
	IDC EC + SPc	1	y = 0.303 + 0.588x	0.305 (0.034–0.658)	0.166 (4)	
	IDC EC	2	y = 0.247 + 0.837x	0.507 (0.209–0.887)	0.232 (4)	4.261
	IDC EC + SPc	2	y = 0.847 + 0.917x	0.119 (0.018–0.249)	0.260 (4)	

P. rapae	IDC EC	1	y = −0.156 + 1.547x	1.261 (0.913–1.795)	1.602 (4)	1.931
	IDC EC + SPc	1	y = 0.250 + 1.349x	0.653 (0.435–0.928)	1.197 (4)	
	IDC EC	2	y = 0.368 + 1.177x	0.486 (0.273–0.737)	1.243 (4)	1.583
	IDC EC + SPc	2	y = 0.664 + 1.295x	0.307 (0.160–0.465)	0.601 (4)	

χ^2 value and degrees of freedom (df) were calculated using the SPSS 27.0 software (SPSS Inc., New York, NY, USA). The efficiency ratio was given as the ratio of IDC EC LC_{50} ÷ IDC EC + SPc formulation LC_{50}.

3.5. SPc Improved the Control Efficacy of Indoxacarb Emulsifiable Concentrate in Field

The DRI and CE of the IDC EC + SPc formulation were higher against both *P. xylostella* (Figure 5A) and *P. rapae* (Figure 5B) than those of IDC EC alone in the field, which is consistent with laboratory bioassay results. For *P. xylostella*, the DRI of the 3,000× IDC EC + SPc formulation was 94.96% at 3 d post application, and its CE was 95.44%. The CE of the 3,000× IDC EC + SPc formulation still remained at 91.16% 7 d after the treatment, which was significantly higher than 82.89% from 3,000× IDC EC alone. SPc increased the CE of IDC EC by up to 8.28% (7d, 3000×). For *P. rapae*, the DRI and CE of the 4000× IDC EC + SPc formulation reached 84.44% and 88.59% at 3 d post application compared to 75.60% and 82.21% in the 4,000× IDC EC treatment. The CE of the 8000× IDC EC + SPc formulation was 71.65% at 3 d after the treatment, which was also higher than 59.13% for 8000× IDC EC alone. SPc increased the CE of IDC EC by up to 12.53% (3 d; 8000×).

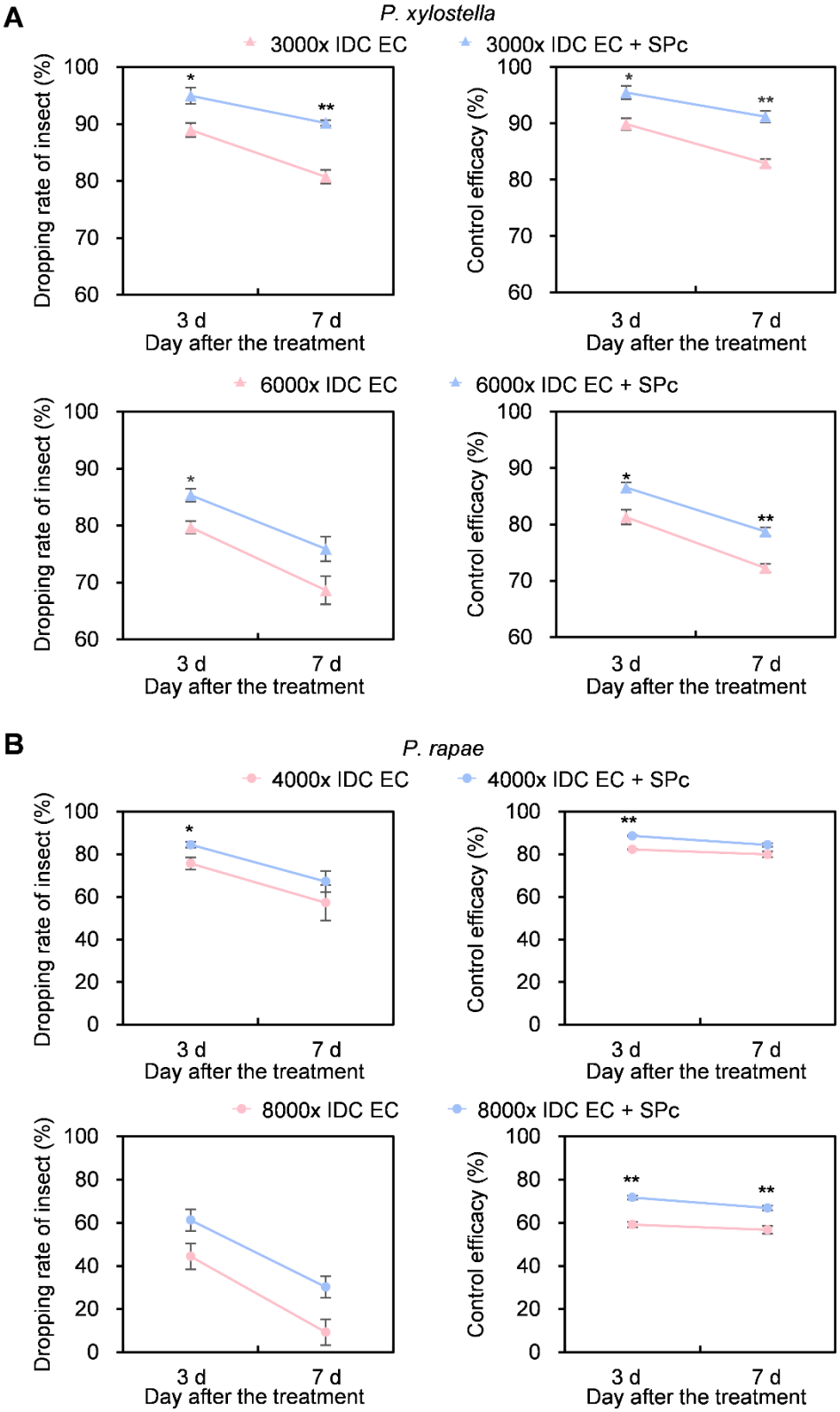

Figure 5. Dropping rate of insect and control efficacy of the IDC EC and IDC EC + SPc formulations at a mass ratio of 1:1 against *P. xylostella* (**A**) and *P. rapae* (**B**). The plot size was 30 m², and each treatment was repeated three times. Ten plants from each plot were randomly selected to record the number of survival larvae at 3 and 7 d post-treatment. "*" and "**" indicate significant differences (independent t-test; $p < 0.05$ and $p < 0.01$).

4. Discussion

In the last decade, a series of publications have reported the successful preparations of nanopesticides based on nanocarriers, which have become a research hotspot [52–54]. Some publications have also confirmed the effectiveness of SPc as an agricultural nanocarrier. SPc can load a variety of pesticides, such as methoxyfenozide [55], imidaclothiz [20], dinotefuran [21], thiamethoxam [56], etc., by simple mixing and incubation. As expected, SPc could also load IDC with a PLC of 18.18%, which was lower than those of methoxyfenozide (32.66%) [55] and thiamethoxam (20.63%) [56] but higher than imidaclothiz (16.31%) [20] and dinotefuran (17.41%) [21]. Moreover, the loading efficiency of SPc toward IDC is superior to that of waterborne polyurethane–sodium alginate nano-emulsion (9.08%), suggesting that SPc is a more cost-effective nanocarrier with high PLC [57].

The self-assembly of the IDC/SPc complex could be attributed to the unique structural characteristics of SPc. The SPc consists of a hydrophilic shell with positively charged tertiary amines and a hydrophobic core [19]. The hydrophilic shell allows SPc to bind with Ais via hydrogen bonding and electrostatic interaction, such as chitosan (hydrogen bonding) and thiamethoxam (electrostatic interaction), which is beneficial for improving the water solubility and dispersion stability of loaded Ais [25,56]. In the current study, the IDC could spontaneously assemble with SPc through hydrophobic interaction due to the hydrophobic core of SPc [18]. Additionally, SPc can assemble with other hydrophobic Ais, such as imidaclothiz and matrine, through hydrophobic interaction [18,20]. The multifaceted binding properties of SPc underscore its potential as a versatile and adaptable nanocarrier for a wide range of applications.

Incorporating nanoparticles into traditional pesticides can reduce the particle size of Ais and improve their solubility in an aqueous solution. For instance, carboxymethyl chitosan-modified carbon nanoparticles can improve the solubility and dispersion stability of emamectin benzoate [58]. The SPc spontaneously assembles with lufenuron, reducing its particle sizes down to 70 nm, respectively [59]. In the current study, the water dispersion of IDC was improved with the aid of SPc, which broke the self-aggregated particles (922 nm) into smaller particles (445 nm). Similarly, other nanocarriers can also reduce the particle size of IDC to the nanoscale, such as HMS-CD (193.26 nm) and ZIF-90-CD (248.57 nm) [45,46]. The reduced particle size of IDC may be attributed to the interaction between nanocarriers and IDC, which disrupts the self-aggregation state of IDC particles.

The off-target loss is a crucial factor contributing to the inefficient application of conventional pesticide formulations. The highly hydrophobic nature of most crop leaf surfaces tends to inhibit liquid deposition, thereby affecting the effective utilization of pesticides. Therefore, leaf hydrophobicity and pesticide droplet retention are key factors influencing pesticide effectiveness. However, reducing pesticide drift and runoff loss on hydrophobic foliage remains a significant challenge [16]. A previous study has demonstrated that the IDC loaded by FL-SiO2 NPs has much better deposition performance on cabbage leaves than IDC alone [44]. Similarly, the retention of IDC EC significantly increased on cabbage leaves with the aid of SPc, indicating that the smaller particle size and higher surface area of the IDC EC + SPc formulation significantly enhanced the adhesion and deposition of droplets on leaves. In line with these findings, Yang et al. [24] have reported that the retention of the proline (Pro)/SPc complex increases from 15.94 to 24.41 mg/cm^2, which is 1.53 times that of Pro alone. Previous studies have also demonstrated that the SPc can reduce the surface tension of pesticide droplets and decrease their contact angle on hydrophobic foliage [16,20,21]. Moreover, the electrostatic interaction between the positively charged SPc and negatively charged leaf surface promotes the adhesion of loaded AIs to plant leaves.

Compared to other insects, most lepidopteran insects can convert IDC into the active metabolite DCJW more rapidly. Therefore, IDC exhibits high selectivity and strong insecticidal activity against lepidopteran insects [60]. The current study demonstrated that the bioactivity and control efficacy of the IDC EC + SPc formulation were remarkably higher than IDC EC against two lepidopteran pests, *P. xylostella* and *P. rapae*. In dose-dependent

experiments, the corrected mortality of *P. xylostella* and *P. rapae* treated with the IDC EC + SPc formulation increased significantly by 26.09% and 21.53%, respectively, with high LC50 efficiency ratios of 6.784 and 1.931. In the field spraying experiments, the addition of SPc to IDC EC resulted in a maximum synergism of 8.28% (3000×, 7d) against *P. xylostella* and 12.53% (8,000×, 3d) against *P. rapae*. These results indicate that SPc has a notable synergistic effect on IDC EC. This is consistent with the findings of Yang et al. [46] and Bilal et al. [44], who have demonstrated the enhanced insecticidal activity of nanocarrier-loaded IDC against insect pests. The potential mechanism for higher bioactivity may involve the small size effect, high adhesion to and deposition on plant leaves, and efficient pesticide delivery to plants and target pests. Compared to IDC EC alone, the SPc-based formulation improves the adhesion and coverage of pests and promotes the translocation of IDC across the insect cuticle [59]. Furthermore, the application of SPc can protect the exogenous agents and activate clathrin-mediated endocytosis for enhanced cellular uptake [25,61]. Notably, when the field application dose is reduced to half of the conventional dose, the efficacy, though not matching that of the conventional dosage, still shows significant improvement with the addition of SPc. This indicates the potential for dosage reduction. Further experiments are needed to quantify the extent to which SPc can reduce the applied IDC EC dosage. Previous studies indicate that SPc is environmentally friendly and highly biocompatible with other beneficial insects [20,23]. Additionally, the current laboratory production cost of SPc is USD 0.16/g [55], which is lower than the cost of commercially available IDC EC of equivalent quality. This cost could be further reduced with large-scale industrial production. Thus, incorporating SPc can decrease the field application rate of IDC EC, reduce environmental impact, and potentially lower pest control costs. Given the significant increase in mortality and control efficacy, we propose that the SPc-based nanodelivery system can be applied as an efficient adjuvant for the design and development of novel pesticides.

5. Conclusions

The current study successfully constructed and developed an SPc-based nanodelivery system for IDC application. The self-assembly of the IDC/SPc complex was primarily driven by hydrophobic interactions, achieving a high loading capacity of 18.18%. This interaction facilitated the formation of the IDC/SPc complex with enhanced dispersibility and reduced particle size. Incorporating SPc into the formulation significantly increased the leaf retention of IDC EC, suggesting that the IDC EC + SPc formulation exhibited superior distribution and spreadability on plant leaves. Consequently, the bioactivity and control efficacy of the IDC EC + SPc formulation were remarkably enhanced against *P. xylostella* and *P. rapae* in both laboratory and field conditions, demonstrating substantial potential for SPc application. The widespread application of the SPc-based nanodelivery system is beneficial for improving pesticide utilization and reducing pesticide application for sustainable agriculture.

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