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Evaluation of a Spinosad Controlled-Release Formulation Based on Chitosan Carrier: Insecticidal Activity against *Plutella xylostella* (L.) Larvae and Dissipation Behavior in Soil

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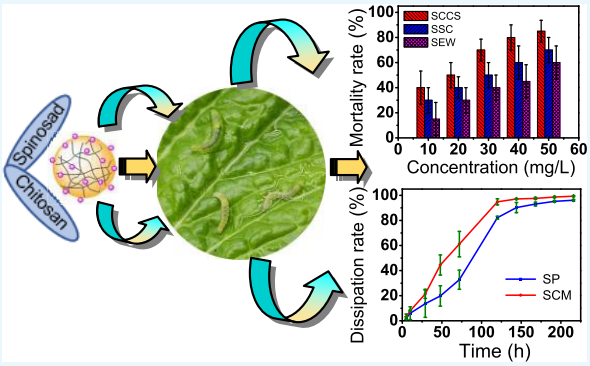
ABSTRACT: Controlled-release pesticide formulations using natural polymers as carriers are highly desirable owing to their good biocompatibility, biodegradability, and improved pesticide utilization. In this study, the application potential of our previously prepared spinosad/chitosan controlled-release suspension (SCCS) was evaluated through both toxicity and dissipation tests. A comparison with the spinosad suspension concentrate and the commercial spinosad emulsion in water showed that the insecticidal activity of SCCS against *Plutella xylostella* larvae displayed the best quick-acting performance as well as long-term efficacy of more than 20 days. The 48 h LC_{50} for a 20-day efficacy was calculated to be 29.36 mg/L. The dissipation behavior of spinosad in the spinosad/chitosan microparticles in soil was found to follow the first-order kinetics, with a relatively shorter half-life (2.1 days) than that observed for the unformulated spinosad (3.1 days). This work showed the positive effect of chitosan on spinosad in improving insecticidal activity and reducing environmental risks in soil, which provided useful information on the application potential of pesticide−carrier systems based on natural polymer materials in crop protection and food safety.



1. INTRODUCTION

Spinosad, mainly composed of spinosyns A and D, is a fermentation product produced by actinomycete *Saccharopolyspora spinosa*.[1] Structurally, the spinosyn family is composed of a central macrolide ring system (aglycon) with rhamnose and forosamine sugars at the 9- and 17-positions, respectively.[2,3] Spinosad is a widely used insecticide featuring high efficacy against Diptera, Thysanoptera, and especially Lepidopteran pests with low toxicity to nontarget organisms.[3] However, shortcomings of conventional spinosad formulations such as easy photolysis or hydrolysis[2,4] result in their repeated and excessive application, which thereby leads to increased environmental risks[3,5] as well as relatively high costs. Recently, sustained/controlled-release formulations of spinosad have shown great potential in reducing side effects of conventional formulations based on the advantages of small effective dose, prolonged release time,[6,7] and particularly specific environmental responsiveness.[8−10]

Natural and biodegradable polymers are usually used as green carriers for pesticide delivery.[11−13] Chitosan, the deacetylation product of natural polymer chitin, has attracted much attention based on its nontoxicity, good biocompatibility, and biodegradability,[14−17] as well as easy modification for adsorption.[18] Especially, the protonation of amino groups in its molecules under acidic conditions makes it possible to be used as a smart molecular device that is sensitive to environmental pH stimulus. Thus, a spinosad/chitosan controlled-release suspension (SCCS) is fabricated through a co-precipitation-based synchronous encapsulation method given in our previous work.[10] The obvious pH and temperature sensitivity, long sustained-release time, and high cumulative release of the formulation are observed through the in vitro release tests. Despite these satisfactory results, primary issues existing in the sustained/controlled-release formulation of pesticides still need to be understood, including (i) What is the actual prevention effect of the formulation against target pests? (ii) Will the formulation exhibit good quick-acting performance or long-acting effect, or both? (iii) Whether the existence of an encapsulation material restrict the dissipation of pesticides, thereby increasing their environmental risk?

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a worldwide destructive pest that can cause severe damage to cruciferous plants.[19−21] The annual worldwide cost for managing *P. xylostella* was estimated to be 1

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Table 1. Physicochemical Properties of SCCS, SSC, and SEW Formulations at 25 °C

formulation	surface tension (mN/m)	viscosity (mPa·s)	contact angle (deg)	pH	density (g/cm^3)
SCCS	38.2 ± 0.1	24.14 ± 2.43	60.05 ± 0.96	10.17 ± 0.01	0.9678 ± 0.0049
SSC	39.2 ± 0.1	60.84 ± 4.79	64.93 ± 0.72	6.52 ± 0.08	1.0078 ± 0.0030
SEW	32.1 ± 0.1	14.24 ± 1.13	48.10 ± 0.78	7.91 ± 0.14	0.9652 ± 0.0086

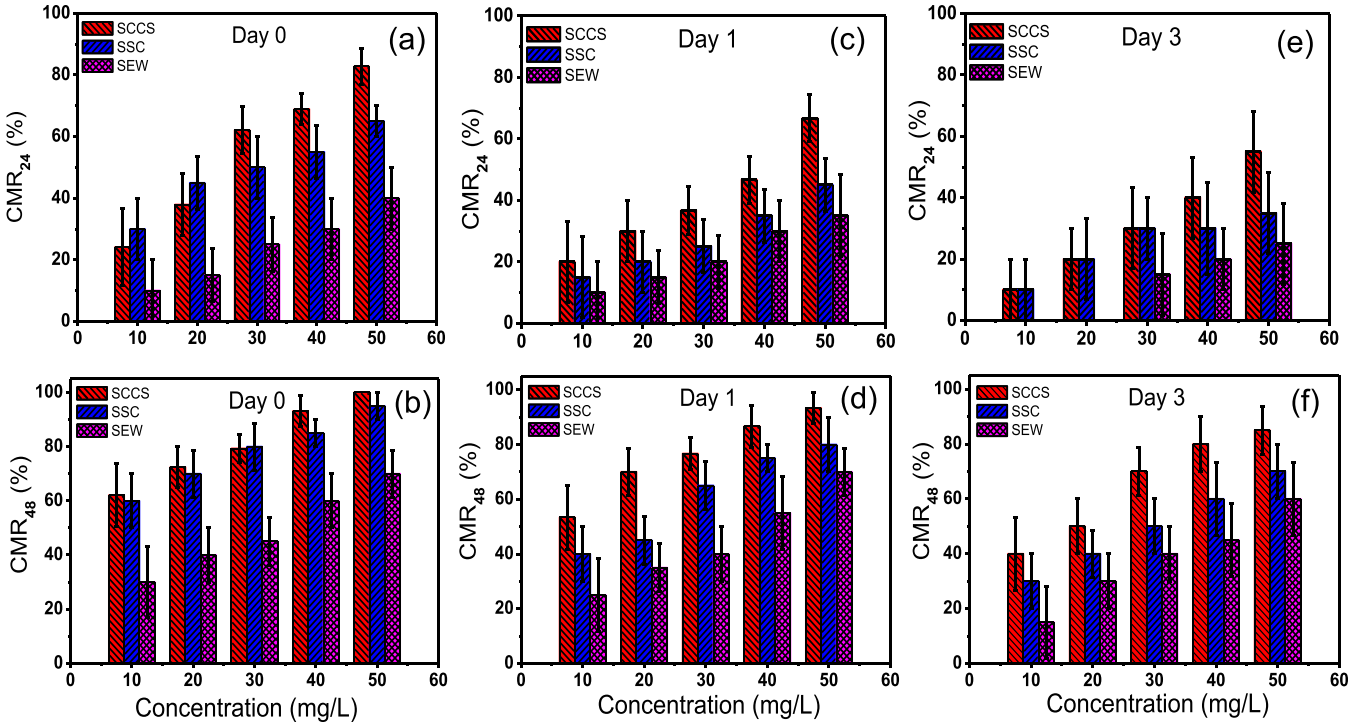

Figure 1. CMR of different concentrations of spinosad formulations against *P. xylostella* larvae 24 and 48 h after toxic leaf feeding, where the Chinese cabbage leaves were picked 2 h (a, b), 1 day (c, d), and 3 days (e, f) after the pesticide treatment. Error bars in this figure represent standard deviation (n = 3).

billion US dollars in 1993,[22] while the total value including yield losses was considered to be 4−5 billion US dollars in 2012.[23] Cabbage family has been proved to be one of the preferred hosts of *P. xylostella*. Several investigations related to the application of spinosad on cabbage plants to control *P. xylostella* have been reported,[24−27] where the direct effectiveness or sublethal effects on the eggs and larvae of *P. xylostella* as well as behavioral differences between the resistant and the susceptible populations were accessed.

Environmental behaviors of pesticide residues associated with their adsorption and degradation in a soil system are important factors that affect the fate of pesticides.[4,28] Thompson et al. observed that the dissipation of spinosad residues in exposed sandy loam soils in central Ontario of Canada followed hyperbolic or exponential decline models.[29] Sharma et al.[30] and Adak et al.[31] also found that the dissipation of spinosad from soil under subtropical conditions obeyed the first-order kinetics. Upon application to the field, the sprayed SCCS is likely to drift to the soil,[32] where both spinosad and the chitosan carrier undergo microbial degradation, chemical hydrolysis, etc. Whether the encapsulation material chitosan plays a protective role in the dissipation of spinosad has become a question worthy of much attention. To the best of our knowledge, there has been no research on the effect of encapsulating materials on the dissipation behavior of spinosad in soil.

In this work, we continue our research on the SCCS to evaluate its toxicity to target species and its dissipation behavior in soil. Using the destructive pest *P. xylostella* as a model, the insecticidal activity of the formulation on Chinese cabbage was examined with the half-lethal concentration calculated. A comparative analysis among the SCCS, the spinosad suspension concentrate (SSC), and the commercial spinosad emulsion in water (SEW) was carried out. Taking the unformulated spinosad as a contrast, the dissipation dynamics of spinosyns A and D in the SCCS in soil were also investigated. The results contribute to the efficacy and environmental risk assessment of the formulation and can provide useful information about its application potential for crop protection and food safety.

2. RESULTS AND DISCUSSION

2.1. Physicochemical Properties. The basic physicochemical properties of SCCS, SSC, and SEW are listed in Table 1, including the surface tension, viscosity, contact angle, pH, and density. As can be seen, the SCCS displayed an alkaline pH of 10.17, with values of surface tension, viscosity, contact angle, and density higher than those of SEW but lower than those of SSC. Interestingly, the contact angle of the SCCS formulation containing 25 mg/L spinosad was measured to be 60.05°, which was very close to the value of 60.47° for the spinosad−polymer nanomicelles on banana leaves.[8] Detailed results of contact angles for different concentrations of

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Table 2. Insecticidal Activity Data of Different Spinosad Formulations against *P. Xylostella* Larvae 24 h after Toxic Leaf Feeding

	time (days)	LC_{50} (mg/L, 95% confidence interval)	LC_{90} (mg/L)	regression equation	regression coefficient
SCCS	0	22.50 (17.83−28.38)	80.46	$y = 1.8689 + 2.3157x$	0.9556
	1	38.28 (28.48−51.45)	234.85	$y = 2.4248 + 1.6268x$	0.8744
	3	50.65 (36.85−69.63)	235.09	$y = 1.7229 + 1.9225x$	0.9665
	14	58.87 (43.44−79.79)	244.26	$y = 1.3292 + 2.0740x$	0.9807
	20	74.10 (53.40−102.82)	323.45	$y = 1.2557 + 2.0025x$	0.9677
SSC	0	27.48 (17.01−44.40)	322.77	$y = 3.2762 + 1.1979x$	0.9690
	1	81.02 (50.46−130.06)	869.22	$y = 2.6265 + 1.2436x$	0.8982
	3	93.49 (58.78−148.69)	934.03	$y = 2.4733 + 1.2821x$	0.9663
SEW	0	86.43 (56.70−131.74)	663.45	$y = 2.1959 + 1.4479x$	0.9566
	1	111.15 (69.68−177.31)	1091.67	$y = 2.3573 + 1.2917x$	0.9476

Table 3. Insecticidal Activity Data of Different Spinosad Formulations against *P. Xylostella* Larvae 48 h after Toxic Leaf Feeding

	time (days)	LC_{50} (mg/L, 95% confidence interval)	LC_{90} (mg/L)	regression equation	regression coefficient
SCCS	0	7.70 (5.62−10.55)	41.73	$y = 3.4528 + 1.7457x$[a]	0.8284
	1	10.05 (7.69−13.14)	47.17	$y = 3.0876 + 1.9084x$	0.9389
	3	15.59 (11.41−21.32)	72.02	$y = 2.6991 + 1.9287x$	0.9461
	14	25.52 (19.14−34.03)	107.04	$y = 2.1044 + 2.0582x$	0.9736
	20	29.36 (20.19−42.69)	195.88	$y = 2.7180 + 1.5548x$	0.9286
SSC	0	8.60 (6.07−12.20)	44.83	$y = 3.3289 + 1.7878x$	0.8633
	1	17.10 (12.00−24.37)	100.62	$y = 2.9473 + 1.6649x$	0.9100
	3	26.06 (17.49−38.83)	198.53	$y = 2.9424 + 1.4532x$	0.9486
	7	44.32 (28.22−69.61)	442.28	$y = 2.8877 + 1.2828x$	0.9749
	10	54.28 (35.49−83.02)	460.84	$y = 2.6067 + 1.3797x$	0.9760
SEW	0	27.13 (18.07−40.72)	214.23	$y = 2.9531 + 1.4280x$	0.8992
	1	31.61 (21.83−45.78)	204.94	$y = 2.6320 + 1.5788x$	0.8726
	3	40.92 (29.01−57.72)	227.62	$y = 2.2279 + 1.7197x$	0.9747

[a]The equation is obtained based on the results of the first four concentrations of spinosad, as CMR for 50 mg/L spinosad reached 100%.

spinosad in the three formulations can be seen in Figure S1. The values for each formulation were less than 65° (with 12.5 mg/L SSC as an exception), indicating the good hydrophilic property of the three formulations.[33]

2.2. Insecticidal Activity. The toxicity test results of different concentrations of SCCS, SSC, and SEW formulations against *P. xylostella* larvae 0, 1, and 3 days after pesticide spraying are shown in Figure 1. Compared with the suspension concentration and the commercial emulsion, the controlled-release formulation of spinosad exhibits the highest CMR against *P. xylostella* larvae 24 and 48 h after toxic leaf feeding. Furthermore, the standard deviations of CMR for the controlled-release formulation are relatively low, indicating the good reproducibility of SCCS in the toxicity tests on *P. xylostella* larvae. At a spinosad concentration of not less than 30 mg/L, CMR_{48} of the SCCS formulation is higher than 79% on day 0 (Figure 1b), 76% on day 1 (Figure 1d), and 70% on day 3 (Figure 1f). All these results indicate that the SCCS formulation prepared in our previous work features a good quick-acting effect against *P. xylostella* larvae. The existence of 40% free spinosad (60% encapsulation efficiency) in the SCCS[10] may play a key role in its quick-acting behavior. In addition, the speed of spinosad release from the carrier chitosan is also considered to be an important factor. Since the main interaction between spinosad and chitosan is physical adsorption and adhesion,[10] spinosad can be released quickly as chitosan chains are gradually broken down in the saliva and intestinal juice of pests. Additionally, high concentrations of chitosan are found to be active against lepidopterous insects, including *P. xylostella* and *Spodoptera exigua* Hübner.[34] Therefore, the good quick-acting performance of this controlled-release formulation can be attributed to the existence of a large number of free spinosad, the relatively quick release of spinosad from chitosan, and the probable synergistic effect of chitosan on the toxicity of spinosad.

As expected, the mortality of larvae caused by the three formulations was time-dependent. The CMR of the three formulations against *P. xylostella* larvae decreased first rapidly and then slowly with the extension of the time interval after pesticide spraying treatment. Compared with the CMR on day 0, the CMR_{24} on day 1 for the three formulations dropped sharply, while the corresponding decline in the values of CMR_{48} was relatively moderate (Figures 1 and S2). Spinosad was able to control the pest through both stomach-poisoning and touch-killing approaches by affecting the nicotinic acetylcholine receptors of their nervous system.[3,35] Meanwhile, it had good penetration capacity in the cabbage leaves. Due to volatilization, photolysism, and penetration of the formulation, the amount of exposed spinosad on the leaf surface on day 1 was much less than that on day 0. Thus, the touch-killing effect on day 1 was reduced dramatically, which resulted in the low values of CRM_{24}. At the same time, the stomach-poisoning effect played a key role in killing the pest at 48 h, giving rise to a relatively moderate decline in the values of CRM_{48}.

Based on the regression equation obtained from the toxicity data fitting, LC_{50} and LC_{90} 24 and 48 h after toxic leaf feeding were calculated and the values are listed in Tables 2 and 3, respectively. The 24 h LC_{50} and LC_{90} of the SCCS formulation

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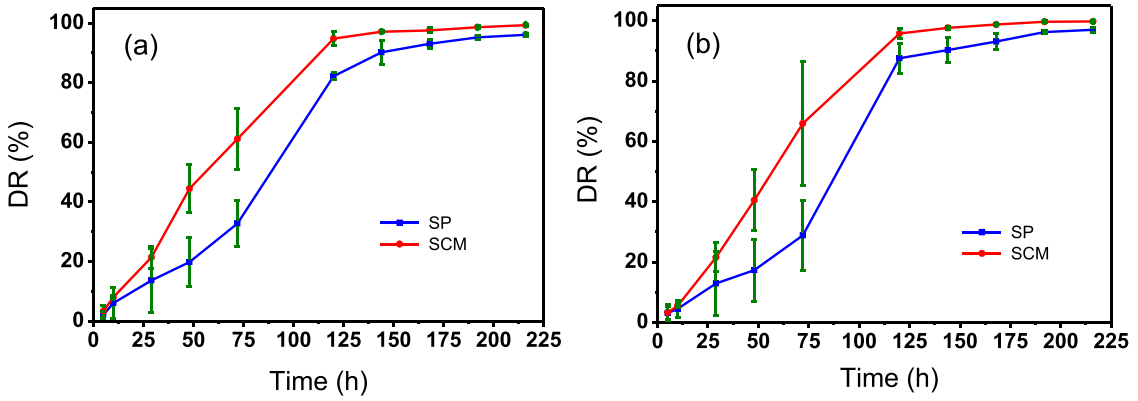

Figure 2. DRs of spinosyn A (a) and spinosyn D (b) in spinosad (SP) and SCM in soil. Error bars in this figure represent standard deviation (n = 4).

on days 0, 1, and 3 were much lower than the corresponding values of the SSC or SEW formulation (Table 2). Compared with the reported data, the 24 h LC_{50} of 22.50 mg/L for the SCCS formulation on day 0 here was much lower than the value of 0.59 g/L for a commercial spinosad formulation (Dow Agro-Sciences, Indianapolis)[24] but relatively higher than the value of 2.265 mg/L for spinosad-sulfamic acid nanoparticles[36] against the second-instar larvae. As the outer wall and wax of the third-instar larvae were thicker than those of the second-instar larvae, it was acceptable that relatively higher LC_{50} was needed for the control of the third-instar larvae. Furthermore, the LC_{50} (22.50 mg/L) of SCCS formulation was 18% lower than the value (27.48 mg/L) of SSC in this work, while the value of 2.265 mg/L for spinosad-sulfamic acid nanoparticles was only 12% lower than the value (2.580 mg/L) of a commercial suspension concentrate. This indicated that the SCCS formulation had a good ability to enhance the insecticidal activity of spinosad. With respect to the 48 h application, the SCCS formulation displayed LC_{50} of 7.70, 10.05, and 15.59 mg/L on days 0, 1, and 3, which were 10, 41, and 40% lower than the values of SSC and 72, 68, and 62% lower than the values of SEW, respectively. The relatively low LC_{50} and LC_{90} of the SCCS formulation in both Tables 2 and 3 confirm its good insecticidal activity against *P. xylostella* larvae.

Based on the sustained-release properties of the SCCS formulation, the long-term efficacy against *P. xylostella* larvae on days 14 and 20 was examined, with CMR of SSC on days 7 and 10 measured for comparison. At spinosad concentrations of 10, 20, 30, 40, and 50 mg/L, the 48 h CMR of SCCS on day 14 were 20, 45, 50, 65, and 75%, corresponding to LC_{50} and LC_{90} of 25.52 and 107.04 mg/L (Table 3), respectively. For the case of day 20 determination, CMR of more than 50% could be achieved at spinosad concentration higher than 40 mg/L. In comparison, the 48 h LC_{50} (29.36 mg/L) of the SCCS formulation on day 20 was found to be lower than the corresponding value (44.32 mg/L) of SSC on day 7 and the value (31.61 mg/L) of SEW on day 1 (Table 3). Obviously, the SCCS formulation displayed the best long-lasting effects. In addition to the synergistic effect of insecticidal activity between chitosan and spinosad, the high encapsulation efficiency of 60% in SCCS and the excellent ultraviolet shielding ability of chitosan[10] were considered to be responsible for its long-term efficacy.

2.3. Dissipation Behavior in Soil. Pesticide residues in soil are one of the important issues that cause serious environmental pollution, which continues to pose risks to the ecosystem and human health. Degradation, adsorption, and migration behaviors of pesticides are the key factors influencing the fate of pesticides in soil. The dissipation rates (DRs) of spinosyns A and D in the unformulated spinosad and the controlled-release SCM in soil are shown in Figure 2a,b, respectively. It can be seen that DRs of both spinosyns A and D increase rapidly in the period of 5−120 h, after which the values increase slowly and reach above 96% within 216 h. The DRs of spinosyns A and D in the SCM at 72 h are 61.1 and 66.0%, respectively, indicating that more than half of the spinosad are dissipated within three days. Interestingly, the dissipation of spinosad in the SCM is slightly faster than the unformulated spinosad under the same conditions, revealing that the controlled-release system not only does not restrict the dissipation of spinosad but also enhances its dissipation. This finding is contrary to the slightly prolonged degradation time of the spirotetramat in a controlled-release system of starch−chitosan−calcium alginate.[37] Different from the chemical synthetic insecticide spirotetramat, the naturally derived spinosad contains both rhamnose and forosamine sugars, which are similar to the polysaccharide chitosan in structure. Based on the good biodegradability of chitosan[38,39] and the fast dissipation of spinosad in soil as well their similar structures, there may be a positive synergistic effect between the degradation behavior of chitosan and spinosad by soil microorganisms.

The regression equations for the dissipation of spinosyns A and D in unformulated spinosad and the SCM are listed in Table 4, with the regression coefficient ranging from 0.9547 to 0.9823. This indicates that the dissipation behavior of spinosad in soil followed the first-order kinetics. The $T_{1/2}$ of spinosyns A and D in the SCM were calculated to be 2.1 and 2.1 days,

Table 4. Dissipation Kinetics of Spinosyns A and D from Unformulated Spinosad (SP) and SCM in Soil

	sample	regression equation	$T_{1/2}$ (h)	regression coefficient
spinosyn A	SP	$\ln C_0/C_t = 0.0221t - 0.9776$[a]	76	0.9823
	SCM	$\ln C_0/C_t = 0.0305t - 0.8631$[b]	51	0.9748
spinosyn D	SP	$\ln C_0/C_t = 0.0230t - 1.0101$[a]	74	0.9547
	SCM	$\ln C_0/C_t = 0.0328t - 0.9572$[b]	50	0.9793

[a]The equation is obtained based on the results from 48 to 168 h. [b]The equation is obtained based on the results from 24 to 144 h.

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which are about 1 day shorter than the corresponding values of 3.2 and 3.1 days for the unformulated spinosad, respectively. Actually, the dissipation behavior of spinosad in soil systems can be affected by many factors. Thompson et al. found that the dissipation half-life of spinosad ranges from 2.0 to 7.8 days based on the different matrix and experimental conditions.[29] A half-life of spinosad is 1.87 days in eggplant-planted soil,[32] 2.8 days in subtropical soil,[30] and 3.6−4.1 days in zucchini-planted soil.[40] Compared with these reported data, the half-life of spinosad in SCM is relatively short, suggesting the reduced environment risk of spinosad in the SCCS formulation in soil.

The durability of the pesticide formulation, a measure of the length of time that a pesticide−carrier complex maintains its integrity after application, is considered a key parameter for environmental risk assessment of pesticide formulations.[28,41,42] The value of durability determines whether further decisions regarding both exposure risks and hazard evaluation of pesticide residues are necessary to be made. Different from the common knowledge that the sustained/controlled-release carrier may restrict the dissipation of pesticide, the relatively short durability of SCM in SCCS is obtained in our work. Thus, it can be considered that the fate parameters of spinosad in SCCS are not significantly different from those in SSC or SEW,[41] revealing a good application potential of chitosan-based controlled-release pesticides.

3. CONCLUSIONS

The application potential of our previously prepared SCCS formulation was evaluated through both toxicity and dissipation tests. Compared with the SSC and the commercially available SEW formulation, the prepared SCCS displayed the best control effect against *P. xylostella* larvae, including both excellent quick-acting performance and long-term efficacy of more than 20 days. This can be attributed to the good sustained-release capability and the outstanding ultraviolet shielding ability of chitosan, as well as the probable synergistic effect of chitosan on the insecticidal activity of spinosad. The 48 h LC_{50} for a 20-day efficacy was calculated to be 29.36 mg/L, which was even lower than the corresponding value (44.32 mg/L) of SSC for a 7-day efficacy and the value (31.61 mg/L) of SEW for a 1-day efficacy. The dissipation behavior of spinosad in the SCM in soil obeyed the first-order kinetic equation. A relatively shorter half-life (2.1 days) of SCM than that of the unformulated spinosad (3.1 days) was observed, indicating the positive effect of chitosan on the degradation behavior of spinosad by soil microorganisms.

4. MATERIALS AND METHODS

4.1. Materials. Spinosad (72% spinosyn A and 18% spinosyn D; Figure S3) was purchased from Qilu Pharmaceutical Co., Ltd. (Inner Mongolia, China). Commercially available 3% SEW was supplied by Hunan Nongda Haite Agricultural Chemical Co., Ltd. (Hunan, China). Chitosan with a viscosity-average molecular weight of $(5.2 \pm 0.4) \times 10^5$ and a degree of deacetylation larger than 90% was obtained from Lan-Ji Biotechnology Development Co., Ltd. (Shanghai, China).[43] Dispersants WELL-301 and WELL-303 were provided by Nanping Well Biochemical Scientific and Technological Co., Ltd. (Fujian, China). Sophorolipid was obtained from the Key Laboratory of Marine Chemistry Theory and Technology of Ministry of Education, Ocean University of China. All of the other chemicals used in the work were of analytical reagent grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

4.2. Preparation of SCCS. The controlled-release suspension of spinosad was prepared by the co-precipitation encapsulation technique, where the natural chitosan was used as the encapsulation material. The specific preparation process has been described in detail in our previous work.[10] Briefly, a mixture of spinosad solution (1.5%, in methanol) and chitosan solution (1.0%, in 0.1 M hydrochloric acid) at a volume ratio of 1:3 was shear emulsified with the existence of emulsifier Tween 80 (0.5%), followed by the addition of the mixture of ammonia (3%) and isopropanol (volume ratio of 4:1) in a dropwise manner under stirring to obtain a spinosad/chitosan suspension. The spinosad/chitosan microparticles (SCMs) used for the dissipation tests in soil were obtained by vacuum filtration of the suspension and subsequent drying at 50 °C.

4.3. Preparation of SSC. The aqueous suspension of spinosad was prepared by the wet grinding method according to our previous findings,[44] where sophorolipid aqueous solution at a concentration of 200 mg/L was used as the dispersion medium. The spinosad (2.5%), the mixture of WELL-301 and WELL-303 in a mass ratio of 2:1 (6.0%), and urea (4.5%) were added into the dispersion medium and ground with zirconium beads (d = 1.2 mm) for 2 h. The volume ratio of the grinding medium to material liquid was 2:1, and the xanthan gum (0.25%) was added 5 min before the end of grinding to adjust the viscosity of the suspension. The obtained suspension concentrate displayed a relatively small medium particle size of 5 μm as well as good suspension stability.

4.4. Physicochemical Properties of SCCS, SSC, and SEW. The surface tension was measured by the Du Noüy ring method using a JYW-200 interfacial tension meter (Dingsheng, China). The viscosity was detected with an MCR102 modular compact rheometer (Anton Paar, Austria) at a shear rate of 50 s^{-1}. A DSA25 contact angle measuring instrument (Kruss, Germany) was used for the contact angle examination, where 5 μL pesticide droplets (12.5, 25, and 50 mg/L of spinosad) were added to the surface of the Chinese cabbage leaf. The pH of the formulation was measured using a PB-10 pH meter (Sartorius, Germany), and the density was determined by means of the pycnometer method. All of the experiments were repeated three times and expressed as mean ± standard deviation.

4.5. Insecticidal Activity. Freshly hatched larvae of *P. xylostella* (L.) (Lepidoptera: Plutellidae) were reared at 25 ± 1 °C, relative humidity of 70 ± 5%, and the light−dark cycle of 16−8 h. The Chinese cabbage (*Brassica campestris L. ssp. chinensis* (*L.*) *Makino. var. communis Tsen et Lee*) "Jiaobai" was planted under laboratory conditions and cultivated without any agrochemical treatments. The 40-day-old cabbage was used for the toxicity tests. The toxic effects of SCCS, SSC, and SEW against the third-instar larvae of *P. xylostella* were determined at different spinosad concentrations of 10, 20, 30, 40, and 50 mg/L, respectively. After the cabbages were sprayed with the assigned pesticide formulations, the leaves were collected at sequential periods of 0, 1, 3, 7, 10, 14, and 20 days to feed the larvae. Taking the survival rate of larvae fed fresh leaves (without pesticide treatment) as the control, the corrected mortality rate (CMR) of larvae 24 or 48 h after eating the toxic leaves can be calculated as follows

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$$\mathrm{CMR} = \frac{M_t - M_c}{1 - M_c} \times 100\% \quad (1)$$

where M_t and M_c are the mortality of the insecticide-treated larva and the nontreated control, respectively.

Statistical analysis of the insecticidal activity data was performed with the help of NORMSINV function in Microsoft Excel software, and the median lethal concentration (LC_{50}) or 90% lethal concentration (LC_{90}) of spinosad can be calculated based on the following regression equation[45]

$$y = ax + b \quad (2)$$

where y = NORMSINV (CMR) + 5, representing the probit value of mortality, and x is the logarithmic value of the spinosad concentration. The letters a and b stand for slope and intercept, respectively.

4.6. Dissipation Behavior in Soil. Brown soil samples without a spinosad application history were collected from randomly selected locations of Qingdao Agricultural University (E 120.40°, N 36.33°) at a depth of 0−15 cm. After the rough screening of the plant roots and stones, the samples were ground and sieved with a 1 mm sieve. The controlled-release SCM and the spinosad powder were mixed uniformly with the soil in the mass ratio of 100 mg/kg, respectively. The mixed samples were sprayed with some water (10 mL/kg) and sealed with a plastic wrap at room temperature to maintain the humidity and then stored in the dark to avoid spinosad photodegradation. At different time intervals, about 20 ± 0.05 g of mixed sample was taken out and transferred into a beaker, to which 40 mL of acetonitrile and 3 g of NaCl were added with pH adjusted to 10. After stirring the solution continuously to completely extract the spinosad, 20 mL of the supernatant was taken out and concentrated to dryness with a rotary evaporation concentrator. The obtained residue was subsequently dissolved in 5 mL of methanol for detection by a Thermo Ultimate 3000 HPLC system with an Agilent Eclipse Plus C18 reversed-phase column. Four repeated measurements were carried out. The dissipation rate (DR) of spinosyns A and D in soil can be calculated as follows

$$\mathrm{DR} = (C_0 - C_t)/C_0 \times 100\% \quad (3)$$

where C_0 (mg/kg) is the initial concentration of spinosad before dissipation and C_t (mg/kg) is the concentration of spinosad recovered from the samples at time t (h).

The data of spinosad residues recovered from soil were fitted to the first-order kinetic equation[30]

$$C_t = C_0 \times e^{-kt} \quad (4)$$

where k is for the dissipation rate constant. The half-life ($T_{1/2}$) of spinosad, the time required for decreasing the concentration of spinosad residues to half of their original amounts, can be calculated based on the corresponding regression equation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04853.

Contact angles of spinosad formulations; time-dependent CMR of SCCS and SSC formulations; and high-performance liquid chromatogram of spinosad (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Sparks, T. C.; Crouse, G. D.; Durst, G. Natural products as insecticides: the biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids. *Pest Manage. Sci.* **2001**, *57*, 896−905.

(2) Cleveland, C. B.; Bormett, G. A.; Saunders, D. G.; Powers, F. L.; McGibbon, A. S.; Reeves, G. L.; Rutherford, L.; Balcer, J. L. Environmental fate of spinosad. 1. Dissipation and degradation in aqueous systems. *J. Agric. Food Chem.* **2002**, *50*, 3244−3256.

(3) Santos, V. S. V.; Pereira, B. B. Properties, toxicity and current applications of the biolarvicide spinosad. *J. Toxicol. Environ. Health, Part B* **2020**, *23*, 13−26.

(4) Adak, T.; Mukherjee, I. Investigating role of abiotic factors on spinosad dissipation. *Bull. Environ. Contam. Toxicol.* **2016**, *96*, 125−129.

(5) Mendonça, T. P.; Aquino, J. D.; Silva, W. J.; Mendes, D. R.; Campos, C. F.; Vieira, J. S.; Barbosa, N. P.; Naves, M. P. C.; Campos Júnior, E. O.; Rezende, A. A. A.; Spanó, M. A.; Bonetti, A. M.; Santos, V. S. V.; Pereira, B. B.; Morais, C. R. Genotoxic and mutagenic assessment of spinosad using bioassays with *Tradescantia pallida* and *Drosophila melanogaster*. *Chemosphere* **2019**, *222*, 503−510.

(6) Huang, B. B.; Zhang, S. F.; Chen, P. H.; Wu, G. Release and degradation of microencapsulated spinosad and emamectin benzoate. *Sci. Rep.* **2017**, *7*, No. 10864.

(7) Badawy, M. E.; Taktak, N. E.; Awad, O. M.; Elfiki, S. A.; El-Ela, N. E. A. Evaluation of released malathion and spinosad from chitosan/alginate/gelatin capsules against *Culex pipiens* larvae. *Res. Rep. Trop. Med.* **2016**, *7*, 23−38.
(8) Dong, E.; Yang, Z.; Zhou, C.; Wang, C.; Li, S.; Ouyang, Q.; Kong, L.; He, Z.; Xie, J.; Li, P.; Yang, P. pH-responsive ultrasonic self-assembly spinosad-loaded nanomicelles and their antifungal activity to *Fusarium oxysporum*. *React. Funct. Polym.* **2019**, *141*, 123−132.
(9) Zhou, C.; Yang, Z.; Zhang, L.; Dong, E.; He, Z.; Liu, X.; Wang, C.; Yang, Y.; Jiao, J.; Liu, Y.; Chen, Y.; Li, P. Self-assembled nano-vesicles based on mPEG-NH_2 modified carboxymethyl chitosan-graft-eleostearic acid conjugates for delivery of spinosad for *Helicoverpa armigera*. *React. Funct. Polym.* **2020**, *146*, No. 104438.
(10) Li, G. B.; Wang, J.; Kong, X. P. Coprecipitation-based synchronous pesticide encapsulation with chitosan for controlled spinosad release. *Carbohydr. Polym.* **2020**, *249*, No. 116865.
(11) Neri-Badang, M. C.; Chakraborty, S. Carbohydrate polymers as controlled release devices for pesticides. *J. Carbohydr. Chem.* **2019**, *38*, 67−85.
(12) Mujtaba, M.; Khawar, K. M.; Camara, M. C.; Carvalho, L. B.; Fraceto, L. F.; Morsi, R. E.; Elsabee, M. Z.; Kaya, M.; Labidi, J.; Ullah, H.; Wang, D. Chitosan-based delivery systems for plants: A brief overview of recent advances and future directions. *Int. J. Biol. Macromol.* **2020**, *154*, 683−697.
(13) Sikder, A.; Pearce, A. K.; Parkinson, S. J.; Napier, R.; O'Reilly, R. K. Recent trends in advanced polymer materials in agriculture related applications. *ACS Appl. Polym. Mater.* **2021**, *3*, 1203−1217.
(14) Kean, T.; Thanou, M. Biodegradation, biodistribution and toxicity of chitosan. *Adv. Drug Delivery Rev.* **2010**, *62*, 3−11.
(15) Kashyap, P. L.; Xiang, X.; Heiden, P. Chitosan nanoparticle based delivery systems for sustainable agriculture. *Int. J. Biol. Macromol.* **2015**, *77*, 36−51.
(16) Meena, L. K.; Rather, H. A.; Vasita, R. Single-step fabrication of core−shell microgels for the controlled release of rhBMP-2 and simvastatin to induce osteogenesis. *ACS Appl. Polym. Mater.* **2020**, *2*, 4902−4913.
(17) Ibrahim, S. M.; Bin Jumah, M. N.; Othman, S. I.; Alruhaimi, R. S.; Al-Khalawi, N.; Salama, Y. F.; Allam, A. A.; Abukhadra, M. R. Synthesis of chitosan/diatomite composite as an advanced delivery system for ibuprofen drug; equilibrium studies and the release profile. *ACS Omega* **2021**, *6*, 13406−13416.
(18) Kyzas, G. Z.; Bikiaris, D. N. Recent modifications of chitosan for adsorption applications: A critical and systematic review. *Mar. Drugs* **2015**, *13*, 312−337.
(19) Sapindal, E.; Ong, K. H.; King, P. J. H. Efficacy of *Azadirachta excelsa* vinegar against *Plutella xylostella*. *Int. J. Pest Manage.* **2018**, *64*, 39−44.
(20) Abro, G. H.; Syed, T. S.; Kalhoro, A. N.; Sheikh, G. H.; Awan, M. S.; Jessar, R. D.; Shelton, A. M. Insecticides for control of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Pakistan and factors that affect their toxicity. *Crop Prot.* **2013**, *52*, 91−96.
(21) Filomeno, C. A.; Barbosa, L. C. A.; Teixeira, R. R.; Pinheiro, A. L.; de Sá Farias, E.; de Paula Silva, E. M.; Picanço, M. C. *Corymbia* spp. and *Eucalyptus* spp. essential oils have insecticidal activity against *Plutella xylostella*. *Ind. Crops Prod.* **2017**, *109*, 374−383.
(22) Talekar, N. S.; Shelton, A. M. Biology, ecology, and management of the diamondback moth. *Annu. Rev. Entomol.* **1993**, *38*, 275−301.
(23) Zalucki, M. P.; Shabbir, A.; Silva, R.; Adamson, D.; Liu, S. S.; Furlong, M. J. Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella* (Lepidoptera: Plutellidae): just how long is a piece of string? *J. Econ. Entomol.* **2012**, *105*, 1115−1129.
(24) Legwaila, M. M.; Munthali, D. C.; Obopile, M.; Kwerepe, B. C. Effectiveness of spinosad against diamondback moth (*Plutella xylostella* L.) eggs and larvae on cabbage under Botswana conditions. *Int. J. Insect Sci.* **2014**, *6*, No. IJIS.S12531.
(25) Yin, X. H.; Wu, Q. J.; Li, X. F.; Zhang, Y. J.; Xu, B. Y. Demographic changes in multigeneration *Plutella xylostella* (Lepidoptera: Plutellidae) after exposure to sublethal concentrations of spinosad. *J. Econ. Entomol.* **2009**, *102*, 357−365.
(26) Chandi, A. K.; Singh, G. Locomotory behaviour of susceptible and resistant *Plutella xylostella (Linnaeus)* (Lepidoptera: Plutellidae). *Phytoparasitica* **2017**, *45*, 541−548.
(27) Zhao, J. Z.; Li, Y. X.; Collins, H. L.; Gusukuma-Minuto, L.; Mau, R. F. L.; Thompson, G. D.; Shelton, A. M. Monitoring and characterization of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad. *J. Econ. Entomol.* **2002**, *95*, 430−436.
(28) Kong, X. P.; Zhang, B. H.; Wang, J. Multiple roles of mesoporous silica in safe pesticide application by nanotechnology: A review. *J. Agric. Food Chem.* **2021**, *69*, 6735−6754.
(29) Thompson, D. G.; Harris, B. J.; Buscarini, T. M.; Chartrand, D. T. Fate of spinosad in litter and soils of a white spruce plantation in central Ontario. *Pest Manage. Sci.* **2002**, *58*, 397−404.
(30) Sharma, A.; Srivastava, A.; Ram, B.; Srivastava, P. C. Dissipation behaviour of spinosad insecticide in soil, cabbage and cauliflower under subtropical conditions. *Pest Manage. Sci.* **2007**, *63*, 1141−1145.
(31) Adak, T.; Mukherjee, I. Dissipation kinetics of spinosad from tomato under sub-tropical agro-climatic conditions. *Environ. Monit. Assess.* **2016**, *188*, No. 299.
(32) Zhao, E.; Xu, Y.; Dong, M.; Jiang, S.; Zhou, Z.; Han, L. Dissipation and residues of spinosad in eggplant and soil. *Bull. Environ. Contam. Toxicol.* **2007**, *78*, 222−225.
(33) Vogler, E. A. Structure and reactivity of water at biomaterial surfaces. *Adv. Colloid Interface Sci.* **1998**, *74*, 69−117.
(34) Zhang, M.; Tan, T.; Yuan, H.; Rui, C. Insecticidal and fungicidal activities of chitosan and oligo-chitosan. *J. Bioact. Compat. Polym.* **2003**, *18*, 391−400.
(35) Lan, J.; Wang, Z.; Chen, Z.; Zhang, L.; Zhao, J.; Guan, Q.; Liao, C.; Liu, N.; Han, Q. Identification of the *Aedes aegypti* nAChR gene family and molecular target of spinosad. *Pest Manage. Sci.* **2021**, *77*, 1633−1641.
(36) Tian, Y.; Tang, G.; Li, Y.; Zhou, Z.; Chen, X.; Gao, Y.; Niu, J.; Yang, J.; Tang, J.; Zhang, Y.; Zhang, X.; Cao, Y. A simple preparation process for an efficient nano-formulation: small molecule self-assembly based on spinosad and sulfamic acid. *Green Chem.* **2021**, *23*, 4882−4891.
(37) Xie, Y. L.; Jiang, W.; Li, F.; Zhang, Y.; Liang, X. Y.; Wang, M.; Zhou, X.; Wu, S. Y.; Zhang, C. H. Controlled release of spirotetramat using starch-chitosan-alginate-encapsulation. *Bull. Environ. Contam. Toxicol.* **2020**, *104*, 149−155.
(38) Wei, X.; Chen, W.; Xiao, M.; Xiao, J.; Wang, Y. Isolation, characterization and application of a chitosan-degrading fungus from soil. *J. Microbiol. Biotechnol.* **2010**, *20*, 1114−1120.
(39) Deshmukh, A. R.; Aloui, H.; Khomlaem, C.; Negi, A.; Yun, J. H.; Kim, H. S.; Kim, B. S. Biodegradable films based on chitosan and defatted *Chlorella* biomass: Functional and physical characterization. *Food Chem.* **2021**, *337*, No. 127777.
(40) Liu, Y.; Sun, H.; Wang, S. Dissipation and residue of spinosad in zucchini under field conditions. *Bull. Environ. Contam. Toxicol.* **2013**, *91*, 256−259.
(41) Walker, G. W.; Kookana, R. S.; Smith, N. E.; Kah, M.; Doolette, C. L.; Reeves, P. T.; Lovell, W.; Anderson, D. J.; Turney, T. W.; Navarro, D. A. Ecological risk assessment of nano-enabled pesticides: A perspective on problem formulation. *J. Agric. Food Chem.* **2018**, *66*, 6480−6486.
(42) Kah, M.; Walch, H.; Hofmann, T. Environmental fate of nanopesticides: durability, sorption and photodegradation of nano-formulated clothianidin. *Environ. Sci.: Nano* **2018**, *5*, 882−889.
(43) Kong, X. P.; Wang, J.; Wang, C. J.; Wu, X. Basicity, water solubility and intrinsic viscosity of carboxymethyl chitosan as a biofunctional material. *Adv. Mater. Res.* **2012**, *531*, 507−510.
(44) Wang, J.; Zhang, S. S.; Zhang, B. H.; Li, J. Z.; Liang, S. K.; Kong, X. P. Preparation and analysis of spinosad 7.5% SC. *Mod. Agrochem.* **2017**, *16*, 17−20.
(45) Kaziem, A. E.; Gao, Y.; He, S.; Li, J. Synthesis and insecticidal activity of enzyme-triggered functionalized hollow mesoporous silica for controlled release. *J. Agric. Food Chem.* **2017**, *65*, 7854−7864.

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