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Preparation and performance of insect virus microcapsules

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Abstract

Background: Biological pesticides, especially baculovirus, often lose their activity under the influence of external light, temperature, and other changes. This limited the application of them. The present study was aimed to prolong the biological activity and ensure the efficacy of a biological pesticide using microencapsulation technology.

Results: In this study, gelatin/carboxymethylcellulose (CMC)-*Spodoptera litura* nucleopolyhedrovirus microcapsules were prepared. The morphological characteristics, apparent morphology, embedding rate, virus loading, particle size, laboratory virulence, and UV resistance of the microencapsulated virus, were tested. The best conditions for preparing gelatin /CMC-*S. litura* nucleopolyhedrovirus microcapsules include the gelatin/CMC ratio of 9:1, wall material concentration of 1%, core material/wall ration ratio of 1:2, re-condensation pH of 4.67, and curing time of 1 h. The prepared microcapsules of *S. litura* nucleopolyhedrovirus exhibited a good external appearance and spherical shapes with an average particle size of 13 μm, an embedding rate of 62.53%, and a drug loading of 43.87%. The virulence test showed that the microencapsulated virus lost by 2.21 times of its initial activity than the untreated virus. After being treated with field exposure, the gelatin/CMC shell of the microcapsule can better protect the virus in the wild environment.

Conclusion: Microencapsulation improves the tolerance of *S. litura* nuclear polyhedrosis virus to ultraviolet radiation. These results will provide ideas for the research of stable and efficient baculovirus preparations and further promote the application and promotion of environmental friendly biological pesticides.

Keywords: Nucleopolyhedrovirus, Microcapsules, UV resistance

Background

China is the largest producer and consumer of pesticides. Safety, efficacy, and environmental compatibility are the major concerns for pesticides (Xing *et al.,* 2019). Excessive use of pesticides has led to a drug resistance among insect populations (Balabanidou *et al.,* 2018). Pesticide residues in agricultural products eventually lead to environmental pollution and ecological imbalance (Bilal *et al.,* 2019).

Baculovirus, as a microbial insecticide, can spread horizontally and vertically in pest populations (Cory *et al.,* 2015) and has high pathogenicity to insect species (Simon *et al.,* 2004). It has received considerable attention to its characteristics such as strong sustainability, low resistance to pests, harmless to vertebrates, and friendly to the environment. Despite the advantages of using baculovirus, a major limitation of the virus is the need for frequent reapplication under field situations. Exposure to ultraviolet solar radiation (UVB, 280–320 nm) is the most critical factor limiting the persistence of entomopathogenic viruses (Villamizar *et al.,* 2009). Consequently, the formulations that encapsulate viral particles have been a preferred delivery system to minimize activity losses due to solar radiation (Tamez *et al.,* 2002).

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Arthurs et al. (2006) spray-dried lignin-encapsulated formulations of CpGV which provided protection from UV radiation. However, the lignin shell of the prepared lignin microcapsules will dissolve in an aqueous solution within hours. It loses the significance of keeping a protective UV coating on the virus after spray application. Camacho et al. (2015) sprayed drying Eudragit® S100 as a polymer coating to protect viral particles of UV-inactivation. Due to the emulsification method, the pollution of a large amount of oil phase during the preparation process and the complicated impurity removal process make the microcapsule process cost too high (Yan *et al.,* 2020).

Herein, the microcapsules were prepared by following a complex coacervation method and utilizing natural polymer gelatin/gum Arabic as the wall material (Gomez *et al.,* 2018). However, gum Arabic is expensive and has poor stability. Because of its low price and stable properties, carboxymethyl cellulose was used as a substitute for gum Arabic. The negatively charged carboxymethyl cellulose can agglomerate with positively charged gelatin below the isoelectric point to yield microcapsules (Duhoranimana *et al.,* 2018). Aldehydes are the most commonly used curing agent for the composite coacervation method. Unlike previous studies, environment-friendly tea polyphenols as the curing agent was used. The o-phenol groups in polyphenol are oxidized to o-benzoquinone to form a co-product with secondary protein amines. The characteristic covalent bond solidifies the capsule wall of the microcapsule, making the capsule wall denser and stronger. Using gelatin/sodium carboxymethylcellulose (CMC) as the wall material, tea polyphenols as the curing agent, and *S. litura* nucleopolyhedrovirus as the core embedding material, the gelatin/carboxymethylcellulose (CMC)-*S. litura* nucleopolyhedrovirus microcapsules were prepared, and their morphology, particle size, drug loading, and embedding rate were analyzed to determine the optimal conditions for preparing the gelatin/CMC wall materials. By measuring the activity of the virus and comparing the non-embedded, microencapsulated viruses, the factors affect the activity of the virus. The UV tolerance of unencapsulated virus and microencapsulated virus was investigated after outdoor sunlight irradiation, providing ideas for the research of stable and efficient baculovirus preparations, so as to further promote the application and promotion of environmental friendly biological pesticides.

Methods

Materials

Type A gelatin (chemically pure or CP), sodium hydroxide (NaOH) (analytically pure or AP), and carboxymethyl cellulose (AP) were purchased from Shanghai Aladdin Reagent Company. Glacial acetic acid (AP) was obtained from Tianjin Chemical Reagent Factory. Tea polyphenols (CP) were purchased from Hefei Basifu Biotechnology Co., Ltd.

Electronic Analytical Balance, CPA225D, Sartorius AG, Germany. Ultra-Pure Water Preparation System, Ulupure, Sichuan Ultra-Pure Technology Guangdong Branch. Collector Constant Temperature Heating Magnetic Stirrer, DF-101S, Gongyi Yuhua Instrument Co., Ltd. Scanning Electron Microscope, S4800, Hitachi Corporation, Japan. Zeta Particle Size Instrument, 90Plus, Bruker Analytical Instruments, Germany. Freeze Dryer, Alpha 1-2 LD Plus, Christ, Germany. Optical Microscope, CX-41X, Olympus, Japan. High Speed Cryogenic Centrifuge, GI54DS, Ebender China Co. Ltd. UV Spectrophotometer, T6 New Century, Beijing Spectroscopy Instrument Co., Ltd.

Insect rearing and virus

Insects and *S. litura* nucleopolyhedrovirus were obtained from Guangzhou Biological Control Station. Larvae were kept individually in half-ounce plastic containers with a fragment of surface contamination of diet. Inoculated larvae were incubated at 27±1°C and fed with artificial diet until dying due to the infection. Dead larvae were collected, ground in distilled sterile water, and homogenized. The virus liquid was obtained by differential centrifugal method, and then the virus powder was freeze-dried.

Preparation of viral microcapsules

Gelatin and CMC were weighed, wherein the mass ratios of gelatin/CMC were 7:1, 9:1, and 11:1. Deionized water was added, and the solution was stirred at 35 °C until it appeared colorless and transparent. Accurately, 100 ml of the mixed solution was taken, m_0 g of virus dry powder was added, then it was dispersed at 300 r/min for 5 min at 25°C. Then, a 10% acetic acid solution was added drop by drop, while using an optical microscope to observe the encapsulation of the polymer in the solution. When capsules appeared, the addition of the acetic acid solution was stopped, and the reaction was continued for 10 min. Tea polyphenol was added (as curing agent) for curing for several hours. The microcapsule suspension was washed with slow filter paper and clean water, and the wet capsules were obtained. The wet capsules were re-suspended and centrifuged at 1000 r/ min for 10 min, the supernatant was discarded, and the precipitate was freeze-dried to obtain solid microcapsules (yield – m_1 g).

Determination of absorbance of composite condensate

Gelatin/CMC solution was prepared at a concentration of 0.1% (w/v). The solution was placed in a 35 °C water bath, the rotation speed was adjusted to 300 r/min, 10% acetic acid was prepared to adjust the pH value of the solution system, samples were withdrawn at different pH

values, and then the absorbance at 600 nm was measured using an ultraviolet spectrophotometer.

Measurement of zeta potential

Gelatin and CMC solution at a concentration of 0.01% (w/v) was prepared and adjusted to the desired pH value. Then, 2 ml of the solution was withdrawn each time, and a particle size analyzer (90Plus, Bruker Analytical Instruments, Germany) was used to determine the zeta potential of the solution.

Calculation of composite condensate yield

Gelatin/CMC solution at a concentration of 0.1% (w/v) was prepared, and the rotational speed was adjusted to 300 r/ min in a 35 °C aqueous solution. Then, a 10% acetic acid was prepared to adjust the pH value of the solution system, until the system undergoes complex coacervation. When the condensate reaction was continued for 10 min, the system was cooled below 15 °C with ice water. The reaction system was refrigerated and allowed to stand overnight. After centrifuging at a low speed, the sediment was collected from the tube and placed in a freeze dryer for 4 h. The weight was recorded, and the yield was then calculated.

Purification and separation of *S. litura* nucleopolyhedrovirus

Differential centrifugation was used, a suspension was prepared with the wet powder of the virus, then it was centrifuged at 1000 r/min for 5 min, and the precipitate was discarded. The supernatant was centrifuged at 3500 r/min for 20 min, then the precipitate was re-suspended and centrifuged at 3500 r/min for 20 min. Differential centrifugation was repeated several times until the polyhedron suspension showed uniformly light gray. The separated virus was freeze-dried to make a dry virus powder. Then, 0.1 g/ml virus solution was prepared, and 1 ml was diluted to 100 ml. Blood cell counting method was used to calculate the virus concentration P_0 (PIB/ml), wherein $P_0 = \frac{A}{80} \times 4 \times 10^6 \times 100$ (A is the number of virus particles in the 5 middle grids or 80 small grids, 100 is the dilution factor).

Determination of drug loading and embedding rate

The filtrate from microcapsule preparation was reserved, after shaking well, placed in a hemocytometer; the number was counted under a microscope; and the amount of virus in the filtrate was calculated as p_1 (PIB).

The calculation formula of microcapsule drug loading:

$$\mathrm{L}\ (\%) = \frac{m_0 - \left(\frac{P_1}{P_0}\right)}{m_1} \times 100\% \quad (1)$$

The embedding rate calculation formula:

$$\mathrm{EE}(\%) = \frac{m_0 - \left(\frac{P_1}{P_0}\right)}{m_0} \times 100\% \quad (2)$$

Observation of microcapsule morphology

During the formation of microcapsules, a drop of reaction suspension was withdrawn and placed on a glass slide. Using an optical microscope, the appropriate magnification and aperture brightness were adjusted to observe the morphology of the microcapsules.

The microencapsulated viruses and purified polyhedron viruses were withdrawn and diluted with an appropriate amount of ultrapure water to prepare a suspension. The suspension was placed on a clean cover glass; it was dried naturally or dried under an infrared lamp. The distance between the infrared lamp and the sample slide was about 15–20 cm. After baking for 5–10 min, gold was sprayed. Finally, the external morphologies of the microcapsules and polyhedrosis viruses were observed under a scanning electron microscope (EVO 18).

Virulence assays

The 3rd instar larvae were selected as test insects. The larvae were reared in plastic boxes with vent holes and 2 ml of each virus suspension after 10-fold concentration dilution was evenly applied, using a brush on the surface of the feed to surface contamination assays. After the surface was room temperature air dried, 3rd instar larvae were inoculated (20 individuals per box). Based on the drug loading calculation, a series of microencapsulated virus suspensions at the same concentration as the unembedded virus were prepared and diluted. The larvae in the control group were fed by the same amount of sterile water, and the experiment was repeated 3 times in each group. The temperature of the culture room was 25 °C, and the light of that was maintained at 12L:12D. The death of the test insects was observed and recorded every day until all larvae died or pupated. After eating the surface of the feed, a clean feed was used for rearing. Count the deaths and calculate the cumulative mortality rate. LC_{50} (Karber method) was calculated by SPSS software.

Field exposure treatment

Place the Petri dish coated with virus polyhedrons and microencapsulated virus dry powder on a platform without sunshade. As the UV intensity in Guangzhou was as

high as 4000 μw/cm^2 at noon at the maximum, the virus will be rapidly inactivated and cannot be sampled and stored in time, so choose field radiation experiments are conducted from 17:00 in the evening to 8:00 in the morning of the next day. Record the daily weather conditions (Table 1) different from the 0, 2, 4, 6, 8, and 10 d time labels, parallel three times each, take them away, and store them in a refrigerator at 4 °C for testing.

Biological test

The viruses and microencapsulated viruses with field exposure treatment were prepared into suspensions with respective 7.75 × 10^6 PIB/ml, and 1 ml was taken on the artificial feed, and the surface was dried naturally. The temperature of the culture room was 25 °C, and the light of that was maintained at 12L:12D. Insert healthy 3rd *S. litura* larvae in the insect box, and receive 20 larvae per treatment, repeat 3 times, and set distilled water as a blank control in each experiment. After eating the surface feed, change the feed and continue feeding, observe the number of larval mortality rate per day, calculate the cumulative mortality, and correct the mortality.

Statistical analysis

All measurements were performed in triplicates. The results obtained were presented as means. Data were mapped by origin 9.1 software (OriginLab Inc., 2013). Log transformed virus test concentrations (PIB/ml) were regressed on cumulative mortality data adopting Probit analysis to estimate LC50 value at 95% confidence limits (Finney, 1971) using SPSS Base 24.0 software (SPSS Inc., 2016). Mortality in control group was included in the Probit analysis.

Results

Analysis of gelatin/CMC complex coacervation

The microscopic interaction between CMC and gelatin molecules generated the composite condensate. As the pH value of the system solution was changed, the solution underwent macroscopic changes from transparent to turbidity then to precipitation, which was caused by the different colloidal states of the macromolecules in the process. Within a certain range, turbidity was proportional to absorbance, so the turbidity of the solution was expressed in terms of absorbance. Figure 1 shows the absorbance of the CMC/gelatin system changes with pH value. When the addition of the acetic acid solution was started, the system pH was nearly neutral. At this time-point, the electrostatic repulsions of CMC and gelatin molecules were strong, because both contained a large amount of negative charge. The turbidity value was close to 0, and macroscopically, the solution appeared clear and transparent. When the volume of instilled acetic acid solution was increased, the pH value slowly decreased; also, the absolute value of the ζ potential of the gelatin molecules started to decrease, and the positive charge gradually increased. Thus, a small amount of soluble composite condensate was produced, and the turbidity of the system slightly increased, but the solution still appeared transparent macroscopically. Subsequently, the pH value was continued to decrease, and the turbidity of the system slowly increased. As the pH value was continuously reduced, after exceeding a certain threshold pH, the turbidity increased rapidly, which indicated that the system underwent phase separation because the soluble composite condensate was produced before undergoing a structural rearrangement, which formed insoluble composite condensate (Jones *et al.*, 2009). When the turbidity rose to the highest point, the pH was the maximum and the peak turbidity indicated that the electrostatic interaction between CMC and gelatin reached a peak. When the pH value was continued to decrease, the turbidity of the system decreased rapidly, this might be related to the insoluble composite condensate to form a composite condensate with poor fluidity and tight structure. After the system solution

Table 1 Daily weather conditions in the field exposure experiment

Date	Weather	Temperature (°C)			UV intensity (μw/cm^2)		
		18 pm	22 pm	8 am	18 pm	22 pm	8 am
20-7-2019	Clear	30	29	27	856	0	1135
21-7-2019	Clear	31	28	29	972	0	1097
22-7-2019	Overcast	27	26	26	358	1	465
23-7-2019	Clear	32	27	29	736	0	861
24-7-2019	The light rain turned fine	25	26	31	104	0	796
25-7-2019	Clear	29	27	30	785	0	1106
26-7-2019	Clear	30	29	31	893	0	1283
27-7-2019	Sunny to overcast	31	27	28	625	0	326
28-7-2019	Clear	32	30	31	830	0	994
29-7-2019	Overcast to light rain	28	25	25	264	0	97

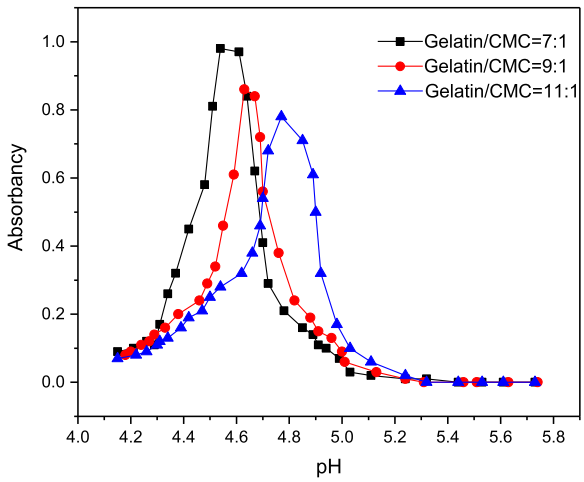

Fig. 1 Variation of absorbance of gelatin/CMC composite condensate with gelatin/CMC ratio and pH

reached a certain pH value, the turbidity decreased slowly, because the aggregation of the protein caused a reduction of the decomposition tendency of the insoluble composite condensate (Serge *et al.*, 2006). The resulting condensate was in a liquid state, which could be evenly dispersed in water. When the pH value was continuously decreased to a certain critical value, the flocculation and sedimentation trends of the condensate were obvious, which might be related to the mutual aggregation of the droplets of the condensate and the denser structure, resulting in a rapid decline in the absorbance value.

The yields of composite condensate with different ratios of gelatin/CMC at different pH values are shown in Fig. 2; when the ratio of gelatin/CMC was 11:1, the yield was the lowest, because the gelatin content was too high, and the content of CMC in the composite condensate

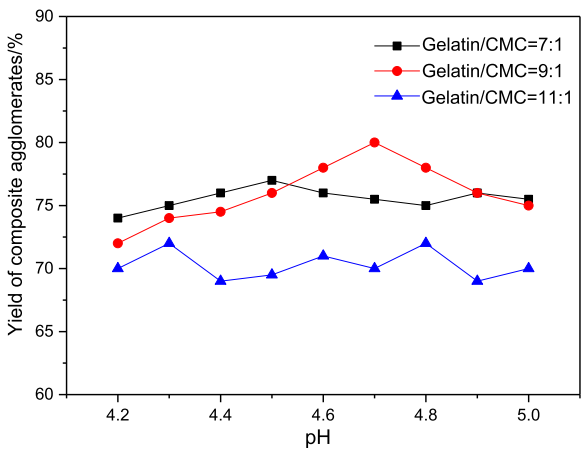

Fig. 2 The impact of gelatin/CMC ratio and pH value on the yields of composite condensates

system was too low, which could not provide sufficient binding sites for the gelatin molecules in the system, then the yield of the composite condensate was reduced. The increase in the yield of the condensate indicated that the utilization rate of the wall material was increased, which was the basis for obtaining a better embedding effect of composite condensate microcapsule. The results of the condensate yield showed that for the three-different gelatin/CMC ratios, the yields of the composite condensate did not change much with the pH value, which also indicated that the change in the amount of the condensate did not cause the absorbance of the colloidal solution in the above figure. The change in absorbance reflected the change in the property of the condensate, caused by pH change.

Determination of wall material ratio and pH value

The CMC/gelatin ratio and pH value determine the properties of the composite condensate as a wall material because the type of charge and the amount of electricity charged by gelatin are greatly affected by the pH change, and the optimal pH value for the coagulation reaction will also be affected by the ratio of CMC and gelatin. Figures 4, 5, and 6 show the morphology of microcapsules prepared at different pH values with the gelatin/CMC ratio from 7:1 to 11:1. In these 3 ratios, as the pH value was changed from low to high, the morphology of the microcapsules was changed from dense super-polymer to single-core microcapsules and small flocculent aggregates.

Figure 3 shows the morphology of microcapsules at different pH values at a gelatin/CMC ratio of 7:1. At pH 4.64, spherical capsules can be produced with uniform particle size; however, the sparse distribution of microcapsule indicates a low yield. A small amount of condensate appeared at pH 4.78 because the surface activity of CMC was low. The quantity of gelatin tape at this time-point was low, and the electrostatic effect was not obvious. At pH 4.51, the microcapsule spheres aggregated with each other to produce agglomerated super-polymer, and the spherical capsules disappeared.

Figure 4 shows the morphology of microcapsules at different pH values when gelatin/CMC was 9:1. The pH range for the formation of spherical microcapsules was 4.54 to 4.71, and its particle size decreased while lowering the pH value. At pH 4.54, the microcapsules were further concentrated and tended to agglomerate. At pH 4.76, the composite condensate formed small strip-shaped agglomerates, because the viscosity of the system was too small, and the wall material did not sufficiently aggregate to form spherical microcapsules. Under this condition, the utilization rate of the microcapsule wall material was low, and it was difficult to be separated by

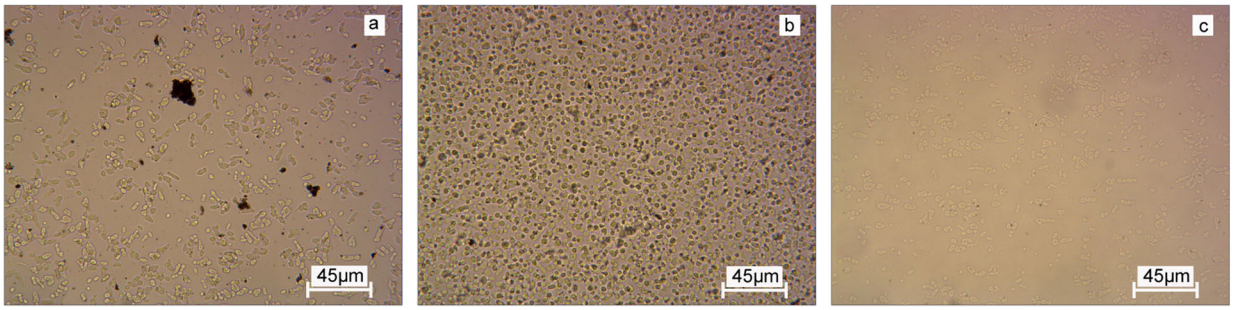

Fig. 3 The morphology of microcapsules at different pH values with the gelatin/CMC ratio of 7:1. a pH= 4.51, b pH= 4.64, c pH= 4.78. When the ratio of gelatin/CMC was 7:1, at pH 4.64, spherical capsules can be produced with uniform particle size; however, the sparse distribution of microcapsule indicates a low yield

filtration after curing, which affected the next research and specific application.

Figure 5 shows the morphology of microcapsules, prepared at different pH values with a gelatin/CMC ratio of 11:1. The pH range of microcapsules for pellet formation was between 4.69 and 4.74, which was narrower than the first 2 ratios, probably because when the ratio of gelatin/CMC was 11:1, the optimal pH value of the prepared microcapsules was close to the isoelectric point of gelatin. At this time-point, the positive charge on the gelatin molecule was small, and it was difficult to form a dense combination with the negatively charged CMC. Besides, the increase in the proportion of gelatin in the system would increase the number of gelatin molecules attached to a single CMC molecule in the formed condensate. Due to the increased steric hindrance, the condensate would become unstable.

Figures 3, 4, and 5 show that, as the ratio of gelatin/CMC increases from 7:1 to 11:1, the pH value, required for a better shape of the microcapsule, gradually increased and the optimal pH value for preparing microcapsule increased with increase in the ratio of gelatin/ CMC. The optimal pH value of the condensation reaction would change, when the ratio of CMC to gelatin was changed, because when the gelatin/CMC ratio was changed, the amount of positive charge in the gelatin molecules could neutralize the negative charge of the CMC, and the pH value at which gelatin molecule carried the positive charge would change accordingly.

The above results suggest that when the ratio of CMC and gelatin is fixed, the morphology and particle size of the composite condensate microcapsules influence pH change. The pH point at the maximum yield of the composite condensate decreased as the protein/polysaccharide ratio was decreased. Compared to gelatin/acacia system, gelatin/CMC system microcapsules were more sensitive to pH changes, the morphology and particle size of gelatin/gum Arabic microcapsules changed significantly, when the pH change was above 0.3 (Sarika *et al.*, 2015), probably because the two colloids carried opposite charges, and the electrostatic force between them drove the complex coacervation. The highest was the proportion of gelatin in the solution system, the greatest was the influence

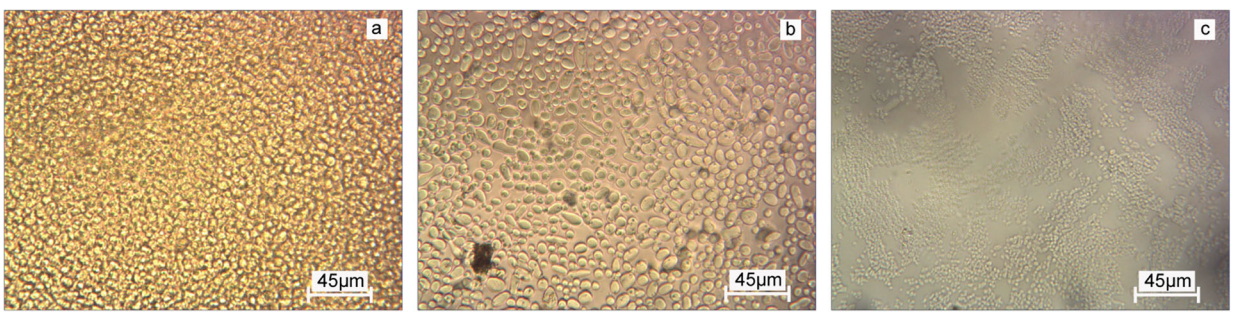

Fig. 4 The morphology of microcapsules at different pH values with the gelatin/CMC ratio of 9:1. a pH= 4.54, b pH= 4.71, c pH= 4.76. When the ratio of gelatin/CMC was 9:1, the pH range for the formation of spherical microcapsules was 4.54 to 4.71, and its particle size decreased while lowering the pH value

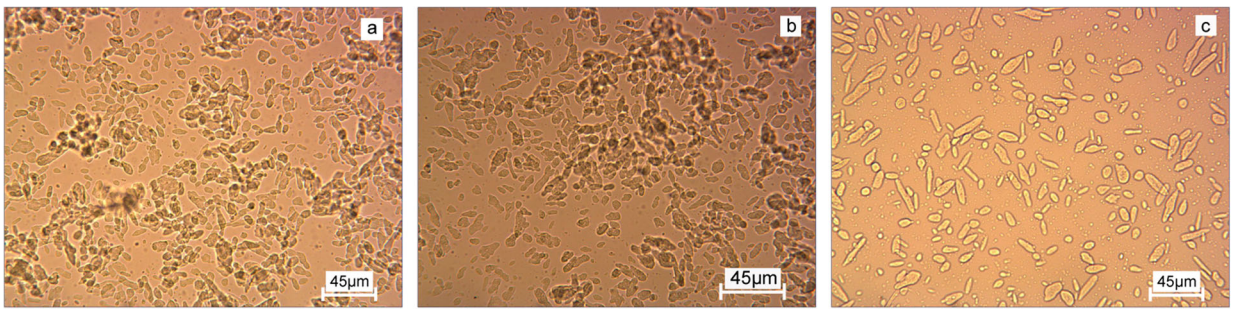

Fig. 5 The microcapsule morphology at different pH values with the gelatin/CMC ratio of 11: 1. a pH= 4.69, b pH= 4.74, c pH= 4.78. When the ratio of gelatin/CMC was 11:1, the pH range of microcapsules for pellet formation was between 4.69 and 4.74

of the total charge of gelatin on the pH value, and the greatest was the influence on the complex coacervation.

Determination of wall material concentration

Figure 6 shows the effect of total wall material concentration on the encapsulation process. Spherical microcapsules appeared, when the wall material concentration was 0.5% (Fig. 7a). However, many empty capsules were not embedded in the core material, because the lowest colloidal concentration in the solution resulted in poor emulsification performance and reduced the stability of the system. The emulsion droplets could easily aggregate to form larger microcapsules, and the density of virus was relatively small in the system. Therefore, many microcapsules were formed with extremely small particle size and empty capsules (without core material), which not only reduced the utilization rate of the wall material, but also the microcapsules with very small particle size eventually clogged the filter paper. Therefore, separation and subsequent operations could not be performed. When the wall material concentration was 1%, the formed microcapsules exhibited uniform particle size and good morphology. When the wall material concentration was increased to 1.5 and 2%, the amount of composite condensate, formed by CMC and gelatin, increased significantly. However, no uniform spherical microcapsule was formed, but huge strip-shaped condensates were produced. Therefore, when the wall material concentration was 1%, the

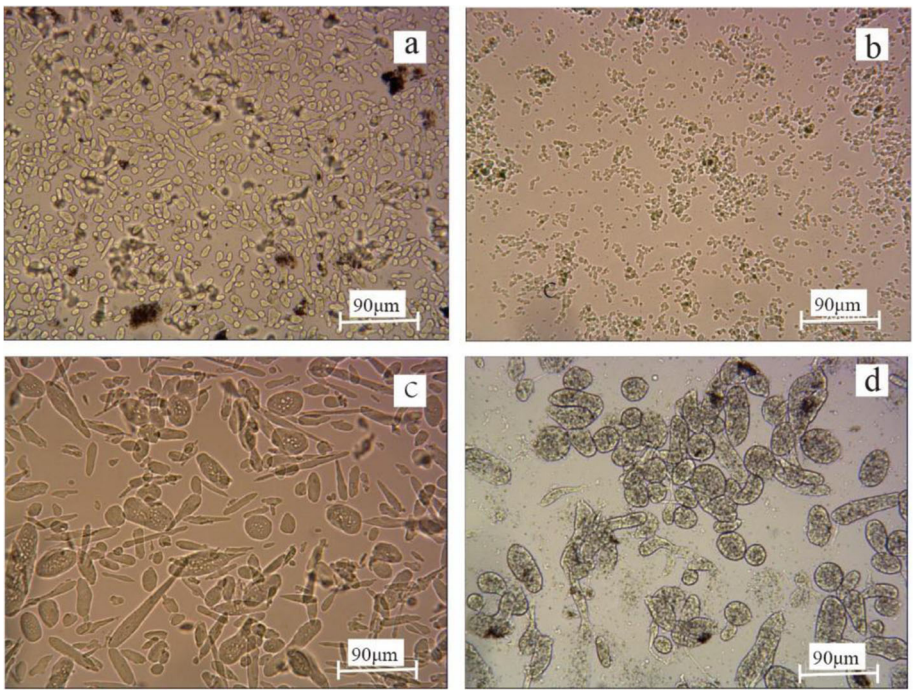

Fig. 6 The effect of wall material concentration on the morphology of composite condensate microcapsules. **a** Wall material concentration was 0.5%. **b** Wall material concentration was 1%. **c** Wall material concentration was 1.5%. **d** Wall material concentration was 2%

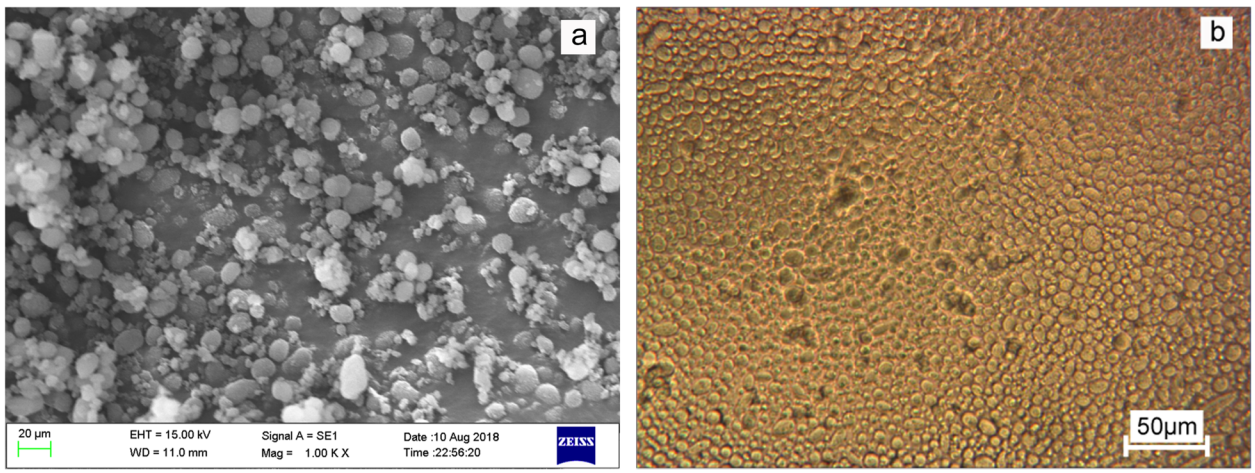

Fig. 7 Observation of microcapsule morphology. **a** Scanning electron micrograph of microcapsules. **b** Optical micrograph of microcapsules

optimal concentration of wall material to form microcapsules was 1%.

Analysis of morphology

Figure 7 shows the scanning electron micrograph and optical micrograph of the virus microcapsules. Figure 7a represents a scanning electron micrograph of virus microcapsules, obtained by solidification and suction filtration, where the spherical microcapsules can be observed clearly; the size is relatively uniform; the surface is smooth and dense; and the microcapsules are adhered to each other, probably because the gelatin in the wet capsule has not been washed thoroughly. Figure 7b represents an optical microscopic image of the virus microcapsule (wet capsules), where the spherical microcapsules with uniform particle size and good dispersion can be observed.

Determination of the core-to-wall ratio

Figure 8 shows that the core-to-wall ratio has not affected the particle size of the microcapsules, probably because the virus particles are smaller than the diameter of the microcapsules, and only a small number of viruses are embedded in a single microcapsule, and the particle size has been affected mainly by the ratio of gelatin/CMC. The core-to-wall ratio affected mainly the drug loading and embedding rate of the virus. Also, the drug-loading amount increased slightly with the

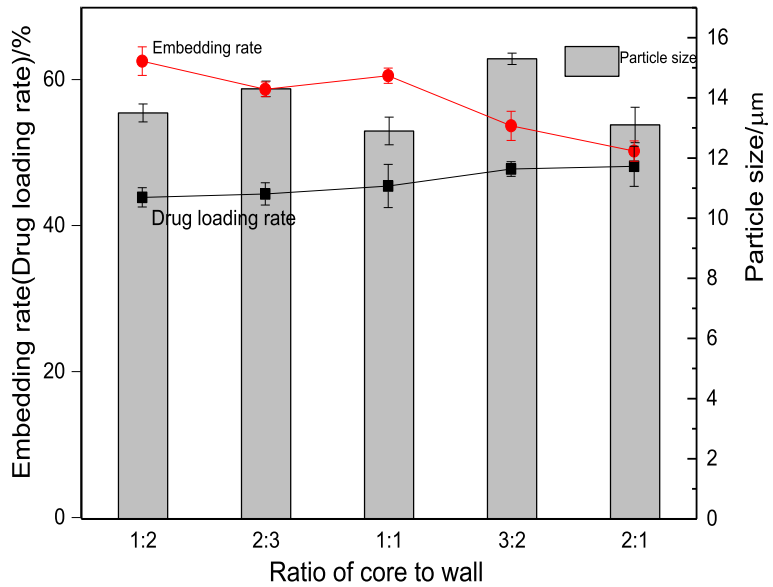

Fig. 8 Effect of core-to-wall ratio on microcapsule parameters

Table 2 Cumulative mortality of 3rd instar larvae of *Spodoptera litura* infected with viruses at different concentrations

Concentration (PIB/ml)	No. of Experimental insects	No. of larval Mortality	Mortality rate (%)	Corrected mortality rate (%)
7.75×10^6	60	58	97	97
7.75×10^5	60	54	90	90
7.75×10^4	60	30	50	50
7.75×10^3	60	10	17	17
7.75×10^2	60	0	0	0
CK	60	0	0	

increase in the core-to-wall ratio, which tended to stabilize. When the core-to-wall ratio was increased from 1:2 to 1:1, the embedding ratio did not change much, and when the core-to-wall ratio was increased from 3:2 to 2:1, the embedding ratio decreased, probably because when the core material concentration in the reaction system was very high, excess core materials could not be embedded, and many unembedded viruses might attach to the surface of the microcapsules and cause the capsule wall to rupture. Therefore, the optimal core to wall ratio was 1:2.

Determination of microencapsulated virus activity

Tables 2, 3, and 4 show the results of virus activity tests. The corrected mortality rate of the microencapsulated virus at all concentrations was lower than that of the unembedded virus, and the LC_{50} value was 8.36×10^4 PIB/ml that was calculated after 192 h of treatment, which was higher than that value of the unembedded virus (1.90×10^4), indicating that the activity of the microencapsulated virus was lower than that of the unembedded virus, probably because the virus was affected by factors, such as the temperature and pH of the reaction system, during the microencapsulation process, causing partial inactivation of the virus. Because the preparation conditions were relatively mild for the virus, the LC_{50} difference between the microencapsulated virus and the unembedded virus was within an order of magnitude. A substantial increase in the resistance of the microencapsulated virus to ultraviolet radiation in subsequent experiments suggested that the level of virus inactivation did not affect the application of microencapsulated virus.

Effect of field exposure on microencapsulated *S. litura* nuclear polyhedrosis virus

The effect of sunlight on the virus is a combination of ultraviolet rays, temperature, and other factors. From Fig. 9, it can be seen that the mortality rate of larvae infected with the virus powder after sunlight exposure from July 20 was only 10%, and the pathogenicity rate of the microencapsulated virus after exposure to the field for 1 day was 53%. After 4 days of exposure in the field, the pathogenicity rate of the virus powder has dropped to 0%, that is, the virus had completely lost its activity. This may be due to the high outdoor ultraviolet intensity in Guangzhou in summer, which has a large inactivation effect on the virus. At the same time, the pathogenicity rate of the microencapsulated virus was 35% on the 4th day, and it remained about 10% after the 10th day. This is because the gelatin/CMC shell of the microcapsule can better protect the virus in the wild environment, reduce the influence of natural factors such as ultraviolet rays on the virus, and thus retain the activity of the virus.

Discussion

S. litura (Lepidoptera: Noctuidae) is a worldwide important agricultural pest, which causes severe damage to cotton, soybeans, tobacco, and other important cash crops (Ahmad et al., 2013), as well as causing severe damage to cash crop in Asia, Africa, South America, and some regions of Oceania (Shad et al., 2012; Tuan et al., 2014). In China, *S. litura* is the most prevalent in the southeast (Su et al., 2012).

The family Baculoviridae comprises 4 genera, of which viruses of the Alphabaculovirus genus (lepidopteran

Table 3 Cumulative mortality rate of 3rd instar larvae of *Spodoptera litura* infected at different concentrations of microencapsulated virus

Concentrations (PIB/ml)	No. of Experimental insects	No. of larval Mortality	Mortality rate (%)	Corrected mortality rate (%)
7.75×10^6	60	51	85	85
7.75×10^5	60	44	73	73
7.75×10^4	60	27	45	45
7.75×10^3	60	5	8	8
7.75×10^2	60	0	0	0
CK	60	0	0	0

Table 4 Regression equation, correlation coefficient, and LC_{50} between the viral tested concentrations and the mortality of 3rd instar larvae

Items	Regression equation	Correlation coefficient	LC50 (PIB/ml)	95 % confidence limit (PIB/ml)	
				Upper limit	Lower limit
Virus	Y = −2.9393 + 1.5215x	0.9381	1.65×10^5	1.20×10^5	2.28×10^5
Microencapsulated virus	Y = −2.44 + 1.34x	0.9240	3.65×10^5	2.53×10^5	5.25×10^5

nucleopolyhedroviruses, NPV) have shown considerable potential as bioinsecticides (Eberle et al., 2012). They are host-specific and have no adverse effect on natural enemies or other non-target insect populations, whereas the application of conventional insecticides reduces the abundance of beneficial agents (Rodgers 1993; Armenta et al., 2003).

Pest control efficacy in the field is often compromised because of the rapid loss of insecticidal activity resulting from exposure to sunlight (Wood and Granados 1991; Huber and Ludcke 1996). Therefore, the development of embedded virus particles has become the preferred delivery system to reduce the loss of activity due to solar radiation (Tamez et al., 2002). Behle et al., (2003) tested spray-dried AfMNPV formulations after storage for insecticidal activity based on bioassays with neonate Trichoplusia ni (Hübner). Experiments demonstrated that AfMNPV in lignin-based spray-dried formulations had a shelf-life of up to 3 months at 30 °C and up to 30 months at 4 °C, and with longer residual insecticidal activity in the field compared to unformulated or a glycerin formulation. Camacho et al. (2015) improved virus survival in ultraviolet light by preparing microcapsules of the virus with Polyacrylic acid resin, but because of its emulsification, the cost of microencapsulation is too high due to the pollution of oil phase and the complicated impurity removal process.

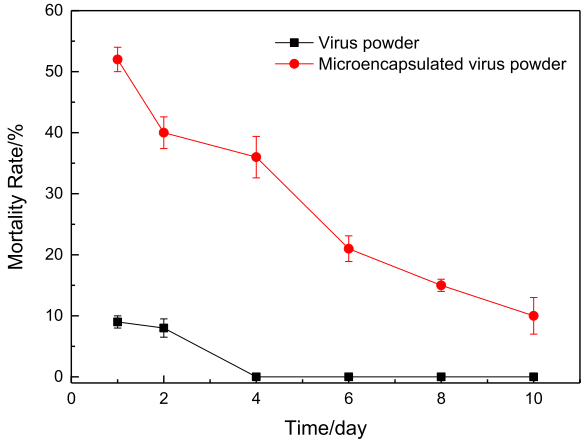

Fig. 9 Infection mortality of *Spodoptera litura* larvae after field exposure

For the sake of prolonging the activity of *S. litura*, microencapsulated NPV was prepared by complex coacervation method. Gelatin/gum Arabic (Yang et al., 2019; Shadel et al., 2018) is usually used to prepare the wall materials of microcapsules. However, gum Arabic is expensive and has poor stability, so CMC, which is cheap and stable, is chosen as the substitute of gum Arabic. The Wall Hardener is formaldehyde (Takenaka et al., 2010)

The wall of the microcapsule was solidified by the covalent bond between o-benzylic phenol and protein secondary amine, and the wall was denser and strongest. However, it can only react under strong alkali condition, and the virus activity will decrease rapidly under alkaline condition. For this reason, the tea polyphenol, which is more green and environmental friendly, is chosen as curing agent to form microcapsules with complete spherical shape, uniform particle size, and good dispersion.

In order to prolong the activity of *S. litura*, the optimum preparation conditions of microcapsules were selected for encapsulation of NPV. The virulence of microencapsulated virus was tested in laboratory and compared to that of unencapsulated virus. The results showed that the corrected mortality of microencapsulated virus was 2.21 times less than that of unencapsulated virus under the same concentration, but it can improve the tolerance of the virus in the field. Consequently, one way to increase baculovirus insecticidal activity is the synergistic combination with low concentrations of synthetic insecticides (Dáder et al., 2020).

Synergy is defined by the interaction of two or more pesticides to produce a combined mortality greater than the sum of their separate effects, which has been shown for azadirachtin and *Helicoverpa armigera* single nucleopolyhedrovirus (HearSNPV), *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV) and *S. litura* multiple nucleopolyhedrovirus (SpltMNPV) (Nathan et al., 2006; Zamora-Avilés et al., 2013), the organophosphate chlorpyrifos and *S. litura* granulovirus (SpltGV) (Subramanian et al., 2005), and the spinosynspinosad and *Spodoptera littoralis* nucleopolyhedrovirus (SpliNPV) and SfMNPV (Méndez et al., 2002; El-Helaly et al., 2015). The mortality of the Guatemalan moth *Tecia solanivora* (Povolný) (Lepidoptera: Gelechiidae) was high when granuloviruses isolated from *Phthorimaea operculella* (Zeller)

(Lepidoptera: Gelechiidae) and *T. solanivora* were combined with the carbamate carbofuram or chlorpyrifos (Espinel et al., 2009).

The combination of baculoviruses and other pesticides in packages is undoubtedly preferable. It is worth noting that the main cause of death is through baculovirus or through the combination of insecticides to kill.

In a natural forest ecosystem, plant chemistries contribute to complex interactions depending on many parameters of the particular ecosystem, such as the density of oak trees in a forest (Elderd et al., 2013). Although comparatively less complex, reports of interactions among crop, pest, and pathogen vary for monoculture cropping systems. The susceptibility of *Helicoverpa zea* to *Helicoverpa zea* nucleopolyhedrovirus was found to be greater when fed on soybean rather than cotton; however, no specific plant chemistry was identified (Ali et al., 1998). In okra and tomato, induced plant defense chemistries are correlated with reduced baculovirus infection of the lepidopteron *Heliothis virescens* (Fabricius); however, induced systemic acquired resistance in cotton foliage had no effect on baculovirus infection of the lepidopteron *H. armigera* (Hübner) (Jeyarani et al., 2011).

Therefore, exploring the interaction among the host plant, beet armyworm, and microencapsulated beet armyworm nucleopolyhedrovirus is also one of the research focuses.

Conclusions

In this study, gelatin/CMC-*S. litura* nucleopolyhedrovirus microcapsules were prepared by the complex coacervation method using *S. litura* nucleopolyhedrovirus as the core material, gelatin, and CMC as the wall materials, and tea polyphenols as the curing agent. The capsule preparation process was simple and cost-effective. The optimal process parameters for the formation of microcapsules were gelatin/CMC ratio of 9:1, wall material concentration of 1%, core wall ratio of 1:2, and curing time of 1 h. The particle size of the prepared virus microcapsules was 13 μm, the drug loading was 43.87%, the embedding rate was 62.53%, and LC_{50} of the microencapsulated virus was 8.36×10^4 (PIB/ml) after 192 h of treatment. The laboratory virulence test of the microencapsulated virus showed that its activity was by 2.21 times less than that of the untreated virus. Sunny and high-temperature weather conditions were not conducive to maintaining the stability of the virus. The activity of *S. litura* nuclear polyhedrosis virus under sunny and high-temperature weather conditions was lower than that under rainy weather conditions. The survival ability of microencapsulated *S. litura* nuclear polyhedrosis virus in the field environment was significantly higher than that of unembedded virus. Microencapsulation improved the tolerance of *S. litura* nuclear polyhedrosis virus to ultraviolet radiation.

Abbreviations

CMC: Carboxymethylcellulose; UV: Ultraviolet solar radiation; GpGv: Cydia pomonella granulovirus

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Authors' contributions

ML prepared the microcapsules and analyzed their surface morphology and field exposure data and was a major contributor to the manuscript. DZ analyzed the virulence assays. Surface potential of microcapsules was measured by JL and CZ. XP and XZ were responsible for writing reviews and edits. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors. All participants have given oral informed consent.

Consent for publication

Not applicable

Competing interests

The authors declare no conflict of interest.

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