Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Capsule-C: an improved *Steinernema carpocapsae* capsule formulation for controlling *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae)

Ziyan NanGong*, Tianhui Li, Weikang Zhang, Ping Song and Qinying Wang

Abstract

Background: Entomopathogenic nematodes (EPNs) have long been used for controlling soil-dwelling insects. *Steinernema carpocapsae* HB310, previously showed a high virulence against many pests including *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae). Due to the lack of durable formulations, up until now, *S. carpocapsae* HB310 has thus far been prevented from use in large-scale farming. The present study aimed to get a better EPNs capsule formulation suitable for long-term storage and effective application.

Results: An improved EPNs capsule formulation, herein named: Capsule-C was prepared by the following composition: Solution I: 18% glycerol, 0.075% formaldehyde, 1% sodium alginate, 0.2% xanthan gum, 0.5% potassium sorbate, 9% glucose, 2% fructose, 2% sucrose, and the remainder was distilled water. The nematodes suspension was added to the alginate mixture in 2×10^4 IJs/mL; Solution II: 18% glycerol, 0.075% formaldehyde, 0.5% calcium chloride, 0.5% potassium sorbate, with the remainder being distilled water. After storage for 180 days at 16 °C and 100% RH, the survival rate of nematodes in Capsule-C was 75.68 ± 0.48% and the nematodes caused 82.33 ± 1.45% mortality in the 5th instar larvae of *Galleria mellonella*. *A. ipsilon* larvae preferred to chew and ingest Capsule-C due to the addition of the glucose compound. The feeding rate of *A. ipsilon* larvae on Capsule-C reached to 100% within 24 h and the larval mortality of *A. ipsilon* was 90.48 ± 6.35%.

Conclusion: EPNs-containing capsules were as effective as sprayed EPNs in water solution at killing *A. ipsilon*. These results will provide ideas to acquire a stable and efficient EPNs capsule formulation and further promote the application of environmental friendly biological pesticides.

Highlights

- We developed an improved capsule formulation named as Capsule-C supplemented with glucose, which can obviously increase the larva feeding amount of *Agrotis ipsilon*.
- The nematodes of *Steinernema carpocapsae* HB310 in Capsule-C survived well and maintained high pathogenicity against *Galleria mellonella* after storage for 180 days.
- Under laboratory conditions, Capsule-C could be released into the rhizosphere directly and effectively reduced maize seedlings damage by *A.ipsilon*.

*Correspondence: ngzyheb@163.com
Affiliation College of Plant Protection, Hebei Agricultural University, Baoding 071001, China

Keywords: *Steinernema carpocapsae*, Calcium alginate capsules, Long-term storage, *Agrotis ipsilon*

Background

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis,* as important candidates, are used as friendly biological pesticides for pest insects in different farming systems, including fruit orchards, turf grass, and greenhouses (Katumanyane et al. 2018). The infective juveniles (IJs) from the Steinernematidae and Heterorhabditidae families target and penetrate the host pests. They release symbiotic bacteria of *Xenorhabdus* and *Photorhabdus*, respectively, which secrete toxic proteins and kill the host insect of initial infection. The IJs then feed on the nutrients of the dead host and multiply. Then, new IJs generation leaves the cadaver in search of new target hosts (Kaya and Gaugler 1993).

EPNs are normally suspended in water and then sprayed on plants or soil. As a living organism, EPNs products still face significant barriers such as the susceptibility to desiccation and Ultraviolet solar (UV) radiation, as well as the lack of durable formulations and appropriate application methods. The inconsistent efficiency of this application method has led to the development of new formulations. Various formulations for EPNs used for below-ground applications include: flowable gel formulations (Leite et al. 2018), calcium alginate granules (Chen and Glazer 2005), attapulgite or bentonite clay (Strauch et al. 2000), water-dispersible granules (Grewal 2000), diatomaceous earth formulations (Kagimu et al. 2019). Notably, the nematodes can also be applied in the formulated host cadavers (Wang et al., 2014). Other works on the formulation of EPNs were focused on above-ground applications by adding surfactants and absorbents (Hiltpold 2015), or mixing EPNs with a surfactant, polymer or insecticide (Guo et al., 2017).

EPNs have a rather short shelf-life and are susceptible to desiccation and UV radiation (Gaugler et al. 1997). These limitations can be overcome by encapsulating EPNs in capsules, which have been shown to provide a humid and UV protective shelter (Vemmer and Patel 2013). The EPNs capsules produced initially were immobilized by a solid core (Kaya and Nelsen 1985). EPNs kept in the calcium alginate granules described by Chen and Glazer (2005) could still cause a 100% host insect mortality after storage for a 6 month period. Additionally, EPNs in alginate gel formulations were reported for having high infectivity against *Corcyra cephalonica* (Umamaheswari et al. 2006) and *Diabrotica virgifera virgifera* (Hiltpold et al. 2012). Moreover, Kim et al. (2015) produced hard capsules by adjusting the capsule properties in order to increase the release time of the nematodes. However, per aforementioned reports these capsules must be dissolved first in water prior to spraying or application in the field.

Black cutworm, *Agrotis ipsilon* (Hufnagel, 1766) (Lepidoptera: Noctuidae), is an agricultural pest of significant economic importance on numerous crops. It feeds on the underground part of the crop, so the effect of chemical control is not ideal (Showers 1997). Biological control may fill the gap left by chemical pesticides and can be used widely to control this insect pest with an environment-friendly mode of action (Behle 2018). *Steinernema carpocapsae* HB310 was found to naturally parasitize the larvae of *A. ipsilon*, which was selected to be embedded in the capsule for this study. In the present study, improved EPNs capsule formulation and evaluation of the infectivity of nematodes in these capsules, following long-term storage were targeted. Mortality of *A. ipsilon* larvae after application with the improved EPNs capsules and comparison it with spraying EPNs suspension was also evaluated.

Methods

Materials

Galleria mellonella L. (Lepidoptera: Pyralidae) and *Agrotis ipsilon* (Huf.) (Lepidoptera: Noctuidae) larvae were obtained from the Pest Biocontrol Laboratory (PBL), Hebei Agricultural University, China. The larvae of *G. mellonella* were reared on an artificial diet (22% maize meal, 22% wheat germ, 11% dried milk, 5.5% dry yeast, 17.5% bee wax, 11% honey, and 11% glycerin) at 29 °C and 70% RH with light conditions (16:8 h L:D). The larvae of *A. ipsilon* were fed on an artificial diet (13% maize meal, 6.5% soybean powder, 5.8% dry yeast, 0.2% sorbic acid, 0.2% methyl-para-hydroxybenzoate, 4.7% vitamin C, 0.2% compound vitamin B, 3.2% sucrose, 1.3% agar, and 64.9% sterilized distilled water) and reared at 29 °C and 70% RH with light conditions (16:8 h L:D).

Steinernema carpocapsae HB310 was obtained from the Pest Biocontrol Laboratory (PBL) at the College of Plant Protection, Hebei Agricultural University. The nematodes were maintained on the 5th instar larvae of *G. mellonella*. Newly emerged IJs from *G. mellonella* cadavers were collected in a White trap (White 1927) and stored using sponges with $2 \times 1 \times 2$ cm dimensions for a maximum of 5 days at 12 °C. They were adapted to 21–23 °C for 24 h before being subjected to the various assays. The pure IJs were suspended in water and prepared in formulations as indicated below.

Zea mays, the host plants were grown in 2 L pots (10 plants per pot) in a greenhouse (24 ± 5 °C) with light conditions (16:8 h L:D) and watered as needed. They were used for the experiments 8–10 days after planting, when they had four fully expanded true leaves.

Survival rate of IJs under different osmotic stresses

In order to find out the best concentration of the glycerol solution used in capsules, survival rate of IJs under different glycerol concentrations was evaluated. First, a sterilized 60% (w/w) glycerol solution and nematode suspensions of 10,000 IJs/mL were prepared. Then, the nematode suspensions and glycerol solution were added to each well of a 24-well plastic plate. The final glycerol concentrations were 8, 12, 18, 24 and 30% (w/w), and the final concentration of the nematodes was 5000 IJs/mL, with 0.075% formaldehyde. Untreated nematodes suspended in distilled water with 0.075% formaldehyde were used as a control treatment. The total volume of the suspension was 1 mL in each well. The plates were incubated at 23 °C for 24 h in order to ensure good mixing of the nematodes with the glycerol solution. Then, 500 µL of the suspension was withdrawn from each well, transferred to a 1.5-mL Eppendorf tube and stored at 23 °C. EPNs produced by *G. mellonella* were used for all the assays. Each treatment was replicated 3 times in 3 separate experimental batches. Next, a 50-µL sample was withdrawn from each treatment and transferred to a 5-cm plastic Petri dish containing 7 mL distilled water. Finally, the survival rate of the nematodes was examined using a stereoscopic microscope.

Encapsulation of EPN in calcium alginate capsules

EPNs in calcium alginate capsules were developed according to previous capsule formulation (Chen and Glazer 2005). First, Solution I, Solution II and the nematodes suspension were prepared. Solution I was designed to choose the best concentration of the glycerol solution from the above experiment, and included 0.075% formaldehyde, 1% sodium alginate, 0.5% potassium sorbate, and the rest was distilled water. Solution II included the best concentration of the glycerol solution, 0.075% formaldehyde, 0.5% calcium chloride, and 0.5% potassium sorbate in distilled water. The nematodes suspension of *S. carpocapsae* HB310 was composed of distilled water and freshly harvested nematodes. Then, IJs of *S. carpocapsae* HB310 were suspended in 20 mL of Solution I (2×10^4 IJs/mL). Calcium alginate capsules with 2-mm diameter were formed by dropping droplets of Solution I into Solution II. The capsules (named as Capsule-A, here) were removed from Solution II with a fine mesh strainer, rinsed with water and stored in 9-cm Petri dishes filled with distilled water. The Petri dishes were stored in 500-mL plastic containers with the lid closed.

During the implementation of the experiment, larvae of *A. ipsilon* could directly ingest Capsule-A. Therefore, the Solution I of other 2 formulations (named as Capsule-B and Capsule-C, respectively) was selected by adding different feeding stimulants based on the original formulation of Capsule-A (Table 1). Among them, Capsule-C was an improved formulation that we developed.

Evaluation the infectivity of nematode in Capsule-C following long-term storage

To examine the impact of the storage on the nematodes in Capsule-C, the plastic containers were placed in an incubator at 16 °C, 100% RH. The nematodes in the control treatments were stored in a sponge with distilled water containing 0.075% formaldehyde at 16 °C and 100% RH. Every month, the survival rate of the nematodes was recorded. One hundred EPN capsules of Capsule-C were

Table 1 Solution I of three capsule formulations adding different feeding stimulants

Kinds	Capsule-A	Capsule-B	Capsule-C
Sodium alginate	1%	1%	1%
Xanthan gum	–	–	0.2%
Linoleic acid	–	0.48%	–
Oleic acid	–	0.24%	–
Glucose	–	3%	9%
Fructose	–	0.4%	2%
Sucrose	–	0.4%	2%
Glycerol	18%	18%	18%
Formaldehyde	0.075%	0.075%	0.075%
Potassium sorbate	0.5%	0.5%	0.5%
Distilled water	Remain	Remain	Remain
Literature	Chen and Glazer (2005)	Hibbard et al. (1994), Bernklau and Bjostad (2008)	Obtained in this paper

transferred to 5-cm plastic Petri dishes that contained 0.2% sodium citrate to dissolve the capsules. After 24 h incubation, the nematode survival rate was determined with a stereoscopic microscope. The test was repeated 3 times.

EPNs capsules of Capsule-C were taken from the plastic Petri dish and dissolved to determine the nematode infectivity recording to the method described by Ricci et al. (1996). The 24-well plastic plates were padded with 2-cm diameter wet filter paper discs to contain humidity. A total of 200 IJs was randomly transferred to each well with a needle under a stereoscopic microscope, and one 5th instar larva of *G. mellonella* was set into each well. Insect corrected mortality was determined after 48 h. The experiments were repeated 3 times, and each treatment was comprised of 60 larvae.

Taxis behavior of *A. ipsilon* on different EPN capsules

In this experiment, taxis behavior of *A. ipsilon* on Capsule-A, Capsule-B and Capsule-C was studied. So, the nematodes were not embedded in each capsule formulation. Fourth instar larvae of *A. ipsilon* were starved for 8 h. In advance. Plastic Petri dishes (15 cm diam.) containing 2 pieces of wet sterile filter paper were prepared. The space of the plastic Petri dishes was subdivided into 4 equal parts with cardboard. Capsule-A, Capsule-B, Capsule-C and maize leaf were placed in the separate compartment with the same weight of 0.3 g, respectively. Ten larvae of *A. ipsilon* were placed in the blank area in the center of Petri dishes and exposed to different EPNs capsules or natural maize leaf. Petri dishes were maintained at 28 °C and 90% RH. The feeding rate of larvae was recorded after 12 h. There were 3 replicate Petri dishes for each treatment and the experiment was conducted 3 times.

Infectivity of capsule-C on the *A. ipsilon*

When the maize host plant had 4 fully expanded true leaves, six 4th instar *A. ipsilon* larvae treated with starvation were carefully placed on surface of the soil. Capsules-C (1000 IJs/larva) were placed into the soil. The IJs suspension was sprayed on the surface of the soil at a dose of 1000 IJs/larva as another treatment. A control group was created and treated with sterile distilled water without IJs. All plots were watered one day before EPNs application and every day thereafter, to provide enough moisture for survival of the nematodes. Pots were covered with parafilm to prevent the larvae from escaping. Pots were maintained in the greenhouse at 28 ± 2 °C and 90% RH. There were 5 replicate pots for each treatment and the experiment was conducted 3 times. Insect corrected mortality was determined after 72 h. Dead larvae were dissected in distilled water and the nematodes were observed under a stereomicroscope to determine the cause of the death.

Statistical analysis

The nematode survival rate, corrected mortality data of *G. mellonella* larvae, feeding rate and corrected mortality data of *A. ipsilon* larvae in the laboratory assay were subjected to analysis of variance with treatment as fixed effect (ANOVA). The significant differences between the treatments were calculated using Tukey's test. All data analyses were performed using SPSS v 16.0 software (SPSS Inc. 2009).

Results

Survival rate of IJs under different osmotic stresses

Survival rate of IJs under different glycerol concentrations was evaluated. When directly exposed to 24 or 30% glycerol solution for 24 h, IJs of *S. carpocapsae* HB310 entered the dormant state quickly, but the survival rate of IJs declined sharply at the first 10 days and dropped down to 0 after 70 days (Fig. 1). The data of 24 or 30% glycerol treatment groups were significantly different from those of CK, 8, 12, and 18% glycerol groups. So, the osmotic pressure of glycerol solution with higher concentration induced IJs to enter dormancy quickly and had a significant effect on IJ survival. When exposed to 8 or 12% glycerol solutions, IJs of *S. carpocapsae* HB310 failed to go into a state of quiescence. So these 2 glycerol concentrations were not suitable for the long time storage of nematodes. For 18% glycerol solution, the *S. carpocapsae* HB310 IJs could maintain the survival rate above 85% after storage for 40 days and kept quiescence for approximately 80 days. Based on these results, the 18% glycerol solution was regarded as the suitable osmotic stress for the next step of EPNs encapsulation.

Composition and preparation of the calcium alginate capsules

Based on the Capsule-A formulation, an improved EPNs capsule formulation (Capsule-C) was composed of the following: Solution I: 18% glycerol, 0.075% formaldehyde, 1% sodium alginate, 0.2% xanthan gum, 0.5% potassium sorbate, 9% glucose, 2% fructose, 2% sucrose, the remainder is distilled water. The nematodes suspension was added to the alginate mixture in 2×10^4 IJs/mL. Solution II: 18% glycerol, 0.075% formaldehyde, 0.5% calcium chloride, 0.5% potassium sorbate, the remainder is distilled water. Capsule-C was transparent sphere particles, 2 mm in diameter similar to Capsule-A and Capsule-B (Fig. 2A). The alginate capsules were stored in Petri dishes, which were stored in 500-mL plastic containers

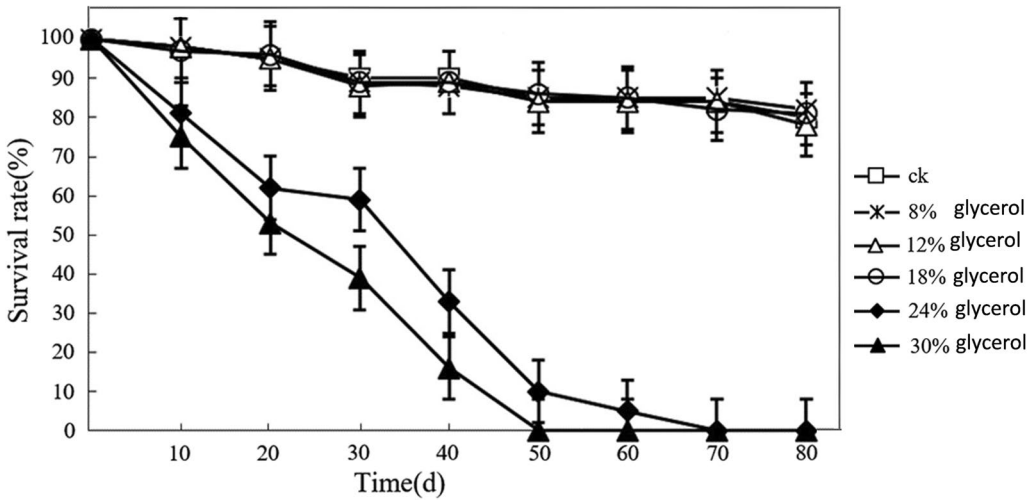

Fig. 1 Effect of glycerol concentration on the survival of *Steinernema carpocapsae* HB310 IJs. CK—IJs stored in distilled water; 8, 12, 18, 24, and 30% glycerol—direct exposure of IJs to concentrations of 8, 12, 18, 24, and 30% glycerol. Values are expressed as the percentage of viable nematodes in the samples obtained from each treatment. The bars represent the standard errors of the means

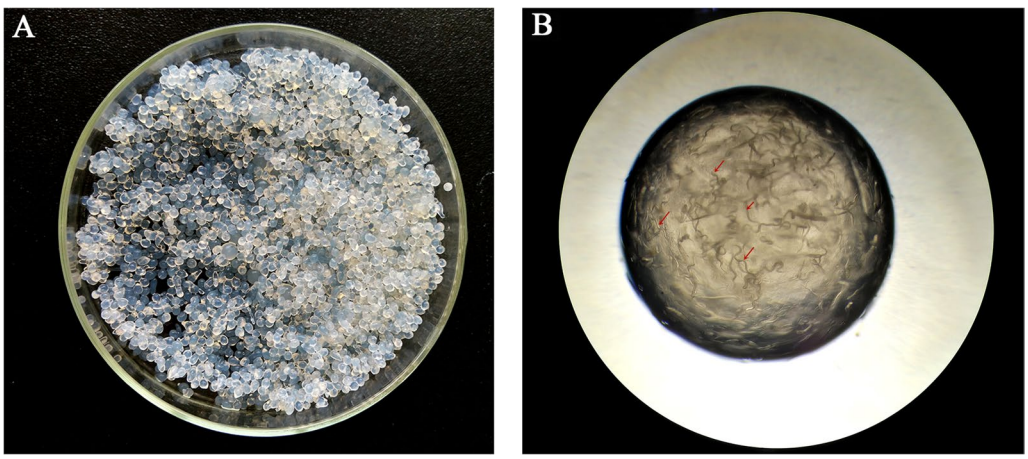

Fig. 2 Photographs of Capsule-C. **A** Alginate capsules in an Ø9-cm Petri dish. Capsule-C products were transparent sphere particles 2 mm in diameter. **B** Morphology of the inactive IJs of *S. carpocapsae* HB310 in Capsule-C. Majority of EPNs entered in a glycerol-induced quiescence. The arrows show some of the IJs that are in dormant

in an incubator at 23 °C and 100% RH. Using a stereomicroscope, majority of EPNs moved to the capsule center within 30 min and after this, most of IJs (>95%) stopped moving and entered in a glycerol-induced quiescence after 48 h (Fig. 2B).

Infectivity of the nematodes in Capsule-C following long-term storage

The survival rate of nematodes in Capsule-C was examined and compared with that of nematodes in the sponge block at 16 °C (Fig. 3A). After dissolving in 0.2% sodium citrate, IJs of *S. carpocapsae* HB310 broke the quiescence and transported into the water in the Petri dishes. The survival rate of the nematodes kept in Capsule-C at 16 °C was 75.68 ± 0.48% after storage for 180 days. The viability of the nematodes kept in the sponge block at 16 °C was gradually reduced, reaching 40.35 ± 2.69% after 180 days. Thus, the survival rate of the nematodes in sponge blocks was lower than that of Capsule-C in the same storage condition.

Further, the infectivity of the nematodes stored in Capsule-C was compared with that of sponge blocks. The nematodes in Capsule-C had a better infectivity on 5th

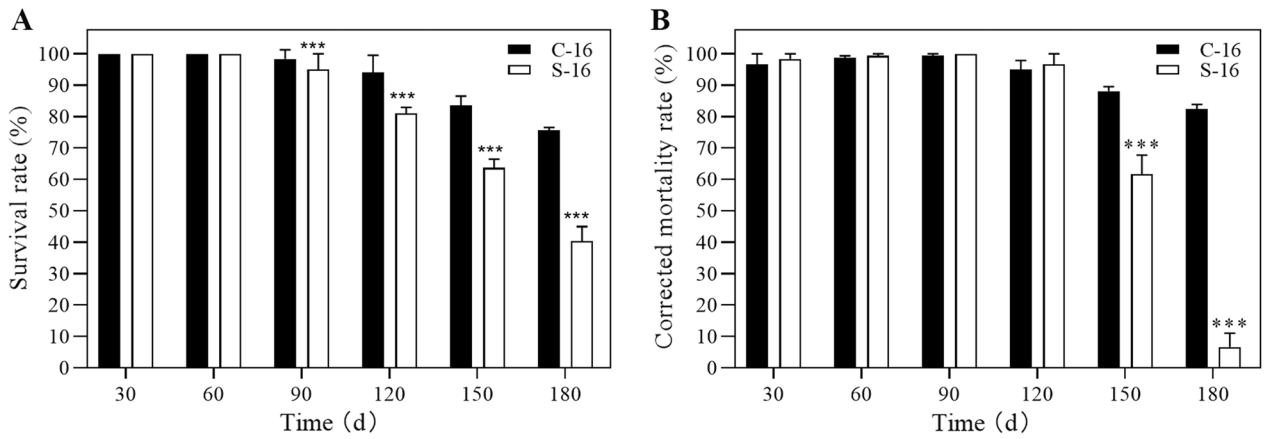

Fig. 3 Lethal effect of *S. carpocapsae* HB310 IJs at different storage conditions. **A** Survival rate of *S. carpocapsae* HB310 IJs stored in the sponge blocks and Capsule-C at 16 °C, 100% RH. **B** Corrected mortality rate of the fifth-instar larvae of *Galleria mellonella* infected by *S. carpocapsae* HB310 IJs stored in the sponge blocks and Capsule-C. C-16: the nematodes in Capsule-C. S-16: the nematodes in the sponge blocks. Bars represent mean percentage ± SE. Probability levels: ***$p < 0.001$

instar larvae of *G. mellonella* according to insect correct mortality (Fig. 3B). After 180 days of storage, the nematodes stored in Capsule-C caused 82.33 ± 1.45% mortality in *G. mellonella,* whereas the nematodes in the sponge blocks caused 6.67 ± 4.41%.

Sponges are the cheapest and most suitable substrates used to store the IJs of EPNs. Then EPNs are normally suspended in water and sprayed onto the soil. But the inconsistent efficiency of this application method had led to the development of new formulations. With this, the preservation effects of the nematodes in the capsules were superior to that of the sponge blocks. EPNs capsules were the favorable application in the future.

Taxis behavior of different EPNs capsules to *A. ipsilon*

Taxis behavior of different EPNs capsules to the test insects was evaluated. Larvae of *A. ipsilon* could directly chew and ingest all the capsules (Fig. 4A). But, *A. ipsilon* larvae preferred to choose Capsule-C (EPNs capsules adding glucose compound) at the same condition

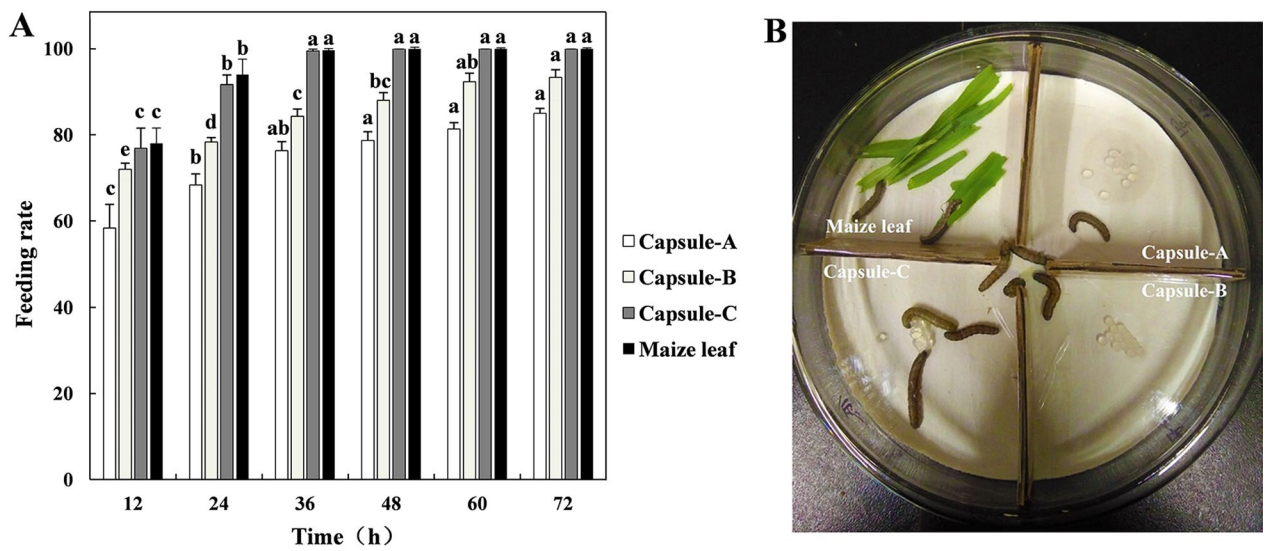

Fig. 4 Taxis behavior of 4th instar larvae of *Agrotis ipsilon* on different EPNs capsules and maize leaf. **A** Feeding rate of *A. ipsilon* larvae on different EPNs capsules and maize leaf. **B** Taxis behavior of *A. ipsilon* larvae on different EPNs capsules and maize leaf. Bars represent mean percentage ± SE. Different letters above bars indicate significant difference among different treatments ($p < 0.05$, Tukey's test)

(Fig. 4B). Within 24 h, *A. ipsilon* larvae almost finished feeding on Capsule-C (Fig. 4A). Therefore, Capsule-C had a better feeding effect on *A. ipsilon* larvae than other capsule formulations.

Infectivity of the nematodes in Capsule-C on the *A. ipsilon*

In the pot assay tests, Capsule-C was directly scattered on the rhizosphere of the maize seedling, comparing the nematodes suspension of *S. carpocapsae* HB310 (Fig. 5A-a). The larval mortalities caused by the nematodes suspension of *S. carpocapsae* HB310 and Capsule-C were 95.24 ± 4.76 and 90.48 ± 6.35%, respectively (Fig. 5B). The larval cadavers infected with nematodes were found in the treatment pots of nematodes suspension and Capsule-C (Fig. 5A-b). In the control pots, the larvae were found feeding on the young stems of maize seedlings and drilling into the soil (Fig. 5A-c). The results were toppling down, wilting and drying of the maize seedlings. The results showed that EPNs Capsule-C was as efficient in reducing maize damage as EPNs suspension.

Discussion

During the complex infection processing, the successful use of EPNs depend on several critical factors, including UV and an adequate environment humidity and temperature (Preisser et al. 2005). Different from other microorganisms biocontrol agents, EPNs are aquatic multicellular organisms, needing enough water for their survival and infectivity. Chen and Glazer (2005) were the first to propose that these problems can be solved by inducing EPNs quiescence with the addition of glycerol to the solution before encapsulation. Guide et al. (2016) concluded that 15% glycerol is effective as cryoprotectant for *Steinernema feltiae*. In this paper, 18% glycerol was regarded as the suitable osmotic stress and moisturizer for *S. carpocapsae* encapsulation. There was enough moisture and aeration for long-term storage of nematodes by keeping EPNs embedded in Capsule-C. The nematodes encapsulated in Capsule-C still had a better infectivity after storage for 6 months. Alginate capsules can improve the nematodes stability and effectiveness as pesticides.

The success of EPNs field applications depends largely on environmental conditions, formulation type, and application techniques (Toepfer et al. 2010). Comparing with the novel formulation methods, EPNs capsules have the potential for a wide range of agricultural applications as carriers of agricultural ingredients for insect pest control (Hoffman 2012). Basis of existing chemical pesticide techniques, the application of EPNs capsules in the field relies on the modification of the existing farm equipment, such as pressurized sprayers and mist blowers (Shapiro-Ilan and Dolinski 2015). But the capsule wall ingredients which were not completely dissolved by sodium citrate will block the device nozzle. During the experiment, an interesting observation is that the host insects could ingest the capsules directly. Bernklau and Bjostad (2008) validated that glucose compound can attract and stimulate feeding effects on western corn rootworm (Coleoptera: Chrysomelidae) larvae. In the present study, an improved capsule formulation added with 9% glucose compound ingredients which can obviously increase the larva feeding amount of *A. ipsilon* was developed.

Kapranas et al. (2020) confirmed that application of EPNs in alginate beads can be more effective than in suspension, most likely because it increases EPNs persistence. Jaffuel et al. (2019) obtained similar results when comparing the application of *Heterorhabditis bacteriophora* in capsules/beads and in suspension to control the western corn rootworm *D. virgifera virgifera* LeConte and the banded cumber beetle *D. balteata* LeConte. Kim et al. (2021) observed that EPNs beads were as effective in reducing root damage by the western corn rootworm (*D. virgifera virgifera*) as that was applied in water in a field trial. In this paper, the nematodes in alginate capsules could be released into the rhizosphere and increase their efficacy. Potential of encapsulated *S. carpocapsae* HB310 in Capsule-C to kill the larvae *A. ipsilon* under laboratory conditions was tested and found that spreading the capsules directly had obvious control effect on *A. ipsilon*. For the future application, it may be more practical to directly bury EPNs capsules, which would be more convenient than conventional EPNs application methods.

Capsule formulation, acting as a proprietary metabolic inhibitor to decreasing nematode oxygen demand, was developed for the storage and transporting of nematodes. All tests in this paper showed encouraging results for the application of the improved EPNs capsules to control *A. ipsilon*. Additional research is necessary to clarify the details of keeping EPNs healthy and infectious in capsules for a longer period. Proper timing of application is critical to ensure an effective protection of the plants, followed by field temperature conditions. So, the researched

(See figure on next page.)

Fig. 5 Protection of the maize seedlings against *A. ipsilon* larvae with Capsule-C. **A**: (a) Display of maize pot assay. (b) The dead larvae of *A. ipsilon* after swallowing Capsule-C. The arrow indicates the cadaver of *A. ipsilon* larvae. (c) Control–maize seedlings without nematode capsules or nematode suspension. The arrow indicates the young stem of maize seedlings chewed by *A. ipsilon* larvae. **B** Mortality of 4th instar larvae of *A. ipsilon* infected by Capsule-C and nematodes suspension. Bars represent mean percentage ± SE. Different letters above bars indicate significant difference among different treatments ($p < 0.05$, Tukey's test)

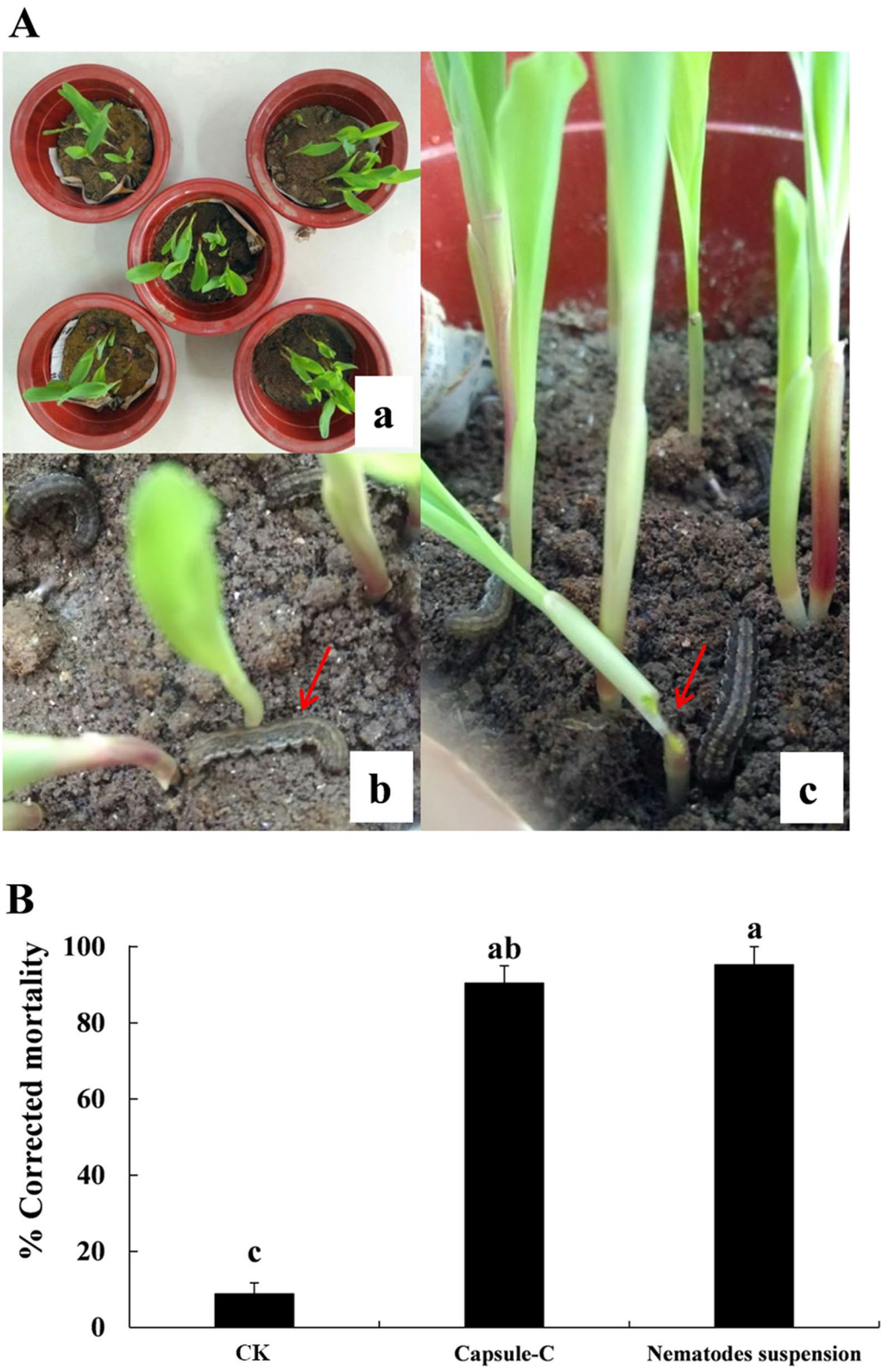

Fig. 5 (See legend on previous page.)

on release time, field temperature and other application technology in the field should be mainly considered in the next step.

Conclusions

In this study, an improved EPNs capsule formulation was prepared using *S. carpocapsae* HB310 as the core material. After storage for 180 days at 16 °C and 100% RH, the survival rate of the nematodes embedding in Capsule-C was 75.68 ± 0.48%. After 180 days of storage, the nematodes stored in Capsule-C caused 82.33 ± 1.45% mortality in *G. mellonella.* The feeding rate of *A. ipsilon* larvae on Capsule-C reached 100% within 24 h. The larval mortality of *A. ipsilon* caused by the nematodes of Capsule-C was 90.48 ± 6.35%. It may be more practical to bury EPNs capsules directly.

Abbreviations

EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; UV: Ultraviolet solar radiation; PBL: Pest Biocontrol Laboratory; RH: Relative humidity.

Acknowledgements

Authors would like to thank the Natural Science Foundation of Hebei Province of China (C2016204107) which supported experimental facilities to carry out experiments.

Authors' contributions

ZNG conceived research and wrote the manuscript. TL prepared the capsules and conducted experiments with WZ. PS analyzed the virulence assays. QW was responsible for writing reviews and edits. All authors read and approved the final manuscript.

Funding

This work was supported by the Natural Science Foundation of Hebei Province of China (C2016204107).

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.

Received: 11 August 2021 Accepted: 15 November 2021
Published online: 27 November 2021

References

Behle RW (2018) In vivo production of *Agrotis ipsilon* nucleopolyhedrovirus for quantity and quality. J Econ Entomol 111:101–107. https://doi.org/10.1093/jee/tox315

Bernklau EJ, Bjostad LB (2008) Identification of feeding stimulants in corn roots for western corn rootworm (Coleoptera: Chrysomelidae) larvae. J Econ Entomol 101(2):341–351. https://doi.org/10.1093/jee/101.2.341

Chen S, Glazer I (2005) A novel method for long-term storage of the entomopathogenic nematode *Steinernema feltiae* at room temperature. Biol Control 32:104–110. https://doi.org/10.1016/j.biocontrol.2004.08.006

Gaugler R, Lewis E, Stuart RJ (1997) Ecology in the service of biological control: the case of entomopathogenic nematodes. Oecologia 109:483–489. https://doi.org/10.1007/s004420050108

Grewal PS (2000) Enhanced ambient storage stability of an entomopathogenic nematode through anhydrobiosis. Pest Manag Sci 56:401–406. https://doi.org/10.1002/(SICI)1526-4998(200005)56:5%3c401::AID-PS137%3e3.0.CO;2-4

Guide BA, Alves VS, Fernandes TAP, Ferreira FP, Neves PMOJ (2016) Glycerol as a cryoprotectant agent to the entomopathogenic nematodes *Heterorhabditis spp*. and *Steinernema spp*. Semin-Cienc Agrar 37:3017–3025. https://doi.org/10.5433/1679-0359.2016v37n5p3017

Guo W, Yan X, Zhao G, Han R (2017) Increased efficacy of entomopathogenic nematode-insecticide combinations against *Holotrichia oblita* (Coleoptera: Scarabaeidae). J Econ Entomol 110:41–51. https://doi.org/10.1093/jee/tow241

Hibbard BE, Bernklau EJ, Bjostad LBL (1994) Long-chain free fatty acids: semiochemicals for host location by western cornrootworm larvae. J Chem Ecol 20:3335–3344. https://doi.org/10.1007/BF02033730

Hiltpold I, Hibbard BE, French BW, Turlings TCJ (2012) Capsules containing entomopathogenic nematodes as a trojan horse approach to control the western corn rootworm. Plant Soil 358:10–25

Hiltpold I (2015) Prospects in the application technology and formulation of entomopathogenic nematodes for biological control of insect pests. In: Campos-Herrera, R. (ed) Nematode pathogenesis of insects and other pests. Springer, Dordrecht, pp 187–205. https://doi.org/10.1007/978-3-319-18266-7_7

Hoffman AS (2012) Hydrogels for biomedical applications. Ann N Y Acad Sci 64:18–23. https://doi.org/10.1111/j.1749-6632.2001.tb03823.x

Jaffuel G, Sbaiti I, Turlings T (2019) Encapsulated entomopathogenic nematodes can protect maize plants from diabrotica balteata larvae. Insects 11(1):27. https://doi.org/10.3390/insects11010027

Kagimu N, Malan AP (2019) Formulation of South African entomopathogenic nematodes using alginate beads and diatomaceous earth. Biocontrol 64(4):413–422. https://doi.org/10.1007/s10526-019-09945-1

Kapranas A, Sbaiti I, Degen T, Turlings T (2020) Biological control of cabbage fly delia radicum with entomopathogenic nematodes: selecting the most effective nematode species and testing a novel application method. Biol Control 144:104212. https://doi.org/10.1016/j.biocontrol.2020.104212

Katumanyane A, Ferreira T, Malan AP (2018) A review of *Bradysia* spp. (Diptera: Sciaridae) as pests in nursery and glasshouse crops, with special reference to biological control using entomopathogenic nematodes. Afr Entomol 26:1–13. https://doi.org/10.4001/003.026.0001

Kaya HK, Gaugler R (1993) Entomopathogenic nematodes. Annu Rev Entomol 38:181–206. https://doi.org/10.1146/annurev.en.38.010193.001145

Kaya HK, Nelsen CE (1985) Encapsulation of *Steinernematid* and *Heterorhabditid* nematodes with calcium alginate: a new approach for insect control and other applications. Environ Entomol 14:572–574. https://doi.org/10.1093/ee/14.5.572

Kim JW, Jaffuel G, Turlings TCJ (2015) Enhanced alginate capsule properties as a formulation of entomopathogenic nematodes. Biol Control 60:527–535. https://doi.org/10.1007/s10526-014-9638-z

Kim J, Hiltpold I, Jaffuel G, Sbaiti I, Hibbard BE, Turlings TCJ (2021) Calcium-alginate beads as a formulation for the application of entomopathogenic nematodes to control rootworms. J Pest Sci. https://doi.org/10.1007/s10340-021-01349-4

Leite LG, Shapiro-Ilan DI, Hazir S (2018) Survival of *Steinernema feltiae*, in different formulation substrates: improved longevity in a mixture of gel and vermiculite. Biol Control 126:192–197. https://doi.org/10.1016/j.biocontrol.2018.05.013

Preisser EL, Dugaw CJ, Dennis B, Strong DR (2005) Long-term survival of the entomopathogenic nematode *Heterorhabditis marelatus*. Environl Entomol 34:1501–1506. https://doi.org/10.1603/0046-225X(2005)034[1501:LSOTEN]2.0.CO;2

Ricci M, Glazer I, Campbell JF, Gaugler R (1996) Comparison of bioassays to measure virulence of different entomopathogenic nematodes. Biocontrol Sci Technol 6:235–245. https://doi.org/10.1080/09583159650039421

Shapiro-Ilan DI, Dolinskil C (2015) Entomopathogenic nematode application technology. In: Campos-Herrera R (ed) Nematode pathogenesis of insects and other pests. Springer, Dordrecht, pp 231–254. https://doi.org/10.3758/s13420-011-0057-z

Showers WB (1997) Migration ecology of the black cutworm. Annu Rev Entomol 42:393–425. https://doi.org/10.1088/0305-4470/39/44/001

Strauch O, Niemann I, Neumann A, Schmidt AJ, Peters A, Ehlers RU (2000) Storage and formulation of the entomopathogenic nematodes *Heterorhabditis indica* and *H-bacteriophora*. Biol Control 45:483–500. https://doi.org/10.1023/A:1026528727365

Toepfer S, Hatala-Zseller I, Ehlers RU, Peters A, Kuhlmann U (2010) The effect of application techniques on field–scale efficacy: Can the use of entomopathogenic nematodes reduce damage by western corn rootworm larvae? Agric for Entomol 12:389–402. https://doi.org/10.1111/j.1461-9563.2010.00487.x

Umamaheswari R, Sivakumar M, Subramanian S (2006) Survival and infectivity of entomopathogenic nematodes in alginate gel formulations against rice meal moth larva, *Corcyra cephalonica Stainton*. Nat Prod Radiance 5:95–98

Vemmer M, Patel AV (2013) Review of encapsulation methods suitable for microbial biological control agents. Biol Control 67:380–389. https://doi.org/10.1016/j.biocontrol.2013.09.003

Wang X, Wang H, Feng QZ, Cui XY, Liu RY, Sun YB, Li GC, Tan H, Song DM, Liu W, Ruan WB, Harvey JA (2014) Desiccation and cold storage of *Galleria mellonella* cadavers and effects on in vivo production of *Steinernema carpocapsae*. Pest Manag Sci 70:895–904. https://doi.org/10.1002/ps.3685

White GF (1927) A method for obtaining infective nematode larvae from cultures. Science 66:302. https://doi.org/10.1126/science.66.1709.302-a

Publisher's Note