Preparation and evaluation of a temperature-sensitive cuelure nano-controlled release agent

Aqiang Wang
Hainan University
Sihua Peng
Hainan University
Bei Zeng
Hainan University
Yuyang Lian
Hainan University
Shuyan Yang
Hainan University
Jingjing Jia
Hainan Academy of Agricultural Sciences (Research Center of Quality Safety and Standards for Agricultural Products of Hainan Academy of Agricultural Sciences)
Bao Wang
Hainan Academy of Agricultural Sciences (Research Center of Quality Safety and Standards for Agricultural Products of Hainan Academy of Agricultural Sciences)
Qiongkuan Zhang
Hainan University
Chengcheng Ni
Hainan University
Mingke Liu
Hainan University
Jie Tao
Hainan University
Junyu Chen
Chinese Academy of Tropical Agricultural Sciences
Qianxing Wu
Hainan University
Xiaofeng Yang
Hainan University
Jianjun Liao
Hainan University

Shihao Zhou

zsh88200939@126.com

Hainan University

Research Article

Keywords: Temperature-sensitive attractant, Zeugodacus cucurbitae Coquillett, Cuelure, Controlled release, Nano-pesticide

Posted Date: December 12th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3718975/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Agronomy on July 19th, 2024. See the published version at https://doi.org/10.3390/agronomy14071578.

Preparation and evaluation of a temperature-sensitive cuelure nano-controlled release agent

Aqiang Wang[1,†], Sihua Peng[1,†], Bei Zeng[1,†], Yuyang Lian[1,†], Shuyan Yang[1,†], Jingjing Jia[2,†], Bao Wang [2,†], Qiongkuan Zhang[1], Chengcheng Ni[1], Mingke Liu[1], Jie Tao[1], Junyu Chen [3], Qianxing Wu[1], Xiaofeng Yang [1], Jianjun Liao [1], Shihao Zhou [1,*]

[1]School of Breeding and Multiplication (Sanya Institute of Breeding and Multiplication), Hainan University, Sanya, Hainan, 572022, China.

[2]Key Laboratory of Plant Disease and Pest Control of Hainan Province/Institute of Plant Protection, Hainan Academy of Agricultural Sciences (Research Center of Quality Safety and Standards for Agricultural Products of Hainan Academy of Agricultural Sciences), Haikou, 571199, China.

[3]Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, 571101, China.

[†]These authors contributed equally.

*Correspondence author, Email: zsh88200939@126.com

Abstract

Cuelure (CUE) is an attractant mainly targeting fruit fly pests and is currently widely used for fruit fly monitoring and control. However, the release speed and time of CUE are not controllable, leading to a large amount of it being wasted, which not only reduces its effectiveness but also increases the cost of pest control. In order to prolong the use time of CUE, improve the utilization rate of the bait, and reduce the cost of pest control. Taking *Zeugodacus cucurbitae* Coquillett as the research object, we develop a temperature-sensitive cuelure nano-controlled release agent, which can control the release rate through the change of environmental temperature. The results show that the temperature-sensitive cuelure nano-controlled release agent not only exhibits good temperature-sensitive controlled release performance, but also shows excellent stability under high-temperature treatment at 60 °C for a week, and the trapping rate of the attractant is 73%-75%. This study not only has great practical significance for the monitoring, early warning, and control of fruit fly pests but also provides a new theoretical basis for the development of insect attractants.

Keywords: Temperature-sensitive attractant; *Zeugodacus cucurbitae* Coquillett; Cuelure; Controlled release; Nano-pesticide

1. Introduction

Zeugodacus cucurbitae Coquillett belongs to Diptera (Tephritidae), which is widely distributed in tropical, subtropical, and temperate countries and regions, mainly affecting cucurbit plants, causing damage to more than 120 species of host plants in 36 families (Chen and Ma, 2010; Ma et al., 2016; Zhou, 2016). The characteristics of *Z. cucurbitae* Coquillett, such as hidden spawning and strong flying ability, lead to the traditional chemical pesticide spraying ineffective. What is more serious is that the extensive use of chemical pesticides will not only bring potential risks to people's food safety but also pollute the environment (Maula et al., 2022). Therefore, in order to mitigate the detrimental effects of synthetic chemical pesticides on human health, food security, and ecosystems, it is vital to embrace pest control strategies that are environmentally friendly.

The emergence of insect attractants offers a novel strategy for controlling pests. By mixing male attractants with pesticides and spraying them on the field in large quantities, the "male-killing" technology of fruit flies, has been utilized to eradicate fruit fly pests on islands isolated from the mainland (Shelly et al., 2014). Cuelure [4-(p-Acetoxyphenyl)-2-butanone] (CUE) is a male attractant for *Z. cucurbitae* Coquillett, which was first discovered in 1960 by Beroza et al. in a series of 4- phenyl -2- butanone isomers synthesized organically (Biswas et al., 2022). It not only enhances the sexual mating activity of male *Z. cucurbitae* Coquillett but also has the ability to attract male *Z. cucurbitae* Coquillett (Guptaa et al., 2022). Meanwhile, CUE also has certain attraction activity to 56 related species, such as *Bactrocera tryoni* Froggatt, *Dacus ciliatus* Low, which are internationally recognized as important attractants for monitoring of fruit flies (Kumaran et al., 2014; Arya et al., 2022; Yazdani et al., 2022).

The occurrence of the pest is particularly serious during the hot season (Li et al., 2023), which is also the optimal time to use CUE to trap *Z. cucurbitae* Coquillett. However, the release speed and time of CUE are subject to various uncertain factors, such as temperature, and it is necessary to replace the core frequently to maintain its effect, which increases the cost of pest control. To avoid pest resurgence, the core must be regularly replaced, resulting in additional pest control costs. Therefore, there is an urgent need to find a material that can react to external triggers such as light, heat, or pH, to manage the speedy and extensive discharge of CUE at a precise temperature, promoting the rate of CUE utilization whilst extending its lifespan.

Poly (N-isopropylacrylamide) (PNIPAM) is a commonly employed hydrogel material with temperature-sensitive properties, with a lower critical solution temperature (LCST) of about 32℃. PNIPAM has been utilized extensively in various domains such as pharmaceuticals, agriculture, and biomedicine due to its super water absorbency, typical three-dimensional porous structure, and excellent responsiveness to environmental stimuli. (Lu et al., 2022; Majstorović et al., 2022; Xiao et al., 2022). Wu et al. (2005) prepared a PNIPAM hydrogel via free radical polymerization. According to their findings, drug release rates and cumulative release increase with temperature. Additionally, the temperature can be used to immediately regulate drug release. Therefore, in this study, PNIPAM hydrogel has been chosen as the switch to control the release of CUE. To enhance both the drug-loading capacity and temperature sensitivity of the PNIPAM hydrogel, we introduced carboxylated multi-walled carbon nanotubes (MWCNTs-COOH) into the hydrogel due to its exceptional drug-loading capacity and thermal conductivity. Carbon nanotubes (CNTs), a material with special structures characterized by a high depth-to-width ratio, high specific surface area, and high mechanical stiffness, can be used as drug containers and have been proven to have a series of potential values (Baydin et al., 2022b;). However, the dispersibility of the carbon nanotube in aqueous solutions is poor, and the introduction of hydrophilic groups carboxyl groups can improve its dispersion in aqueous solutions (Zhang et al., 2022a). Moreover, MWCNTs-COOH not only facilitates targeted delivery of hydrophilic and hydrophobic substances but also maintains drug stability (Zhang et al., 2009).

To sum up, to address the issue of the uncontrollable release rate of insect attractants, we propose to select *Z. cucurbitae* Coquillett as the research object, flytrap ketone as the prodrug, MWCNTs-COOH as the carrier, and PNIPAM hydrogel as the switch to control of the release of the attraction, to develop a temperature-sensitive cuelure nano-controlled release agent (hereinafter referred to as temperature-sensitive attractant), so as to realize the controlled release of CUE, to reduce the cost of the use of the attractant, and to provide the theoretical basis for the monitoring and control of fruit fly pests.

2. Materials and methods

2.1. Insect source

The insect source of *Z. cucurbitae* Coquillett used in this study was collected from a bitter melon plantation in Hainan, China (109°29'E, 19°30'N). The damaged fruits were brought indoors, and the larvae and pupae of *Z. cucurbitae* Coquillett were taken out for artificial breeding. The average indoor temperature is kept at 25±1℃, the humidity is kept at 70±5%, and the light ratio is 14L:10D, so as to establish a temperature-sensitive indoor population. The formula of artificial feed for insect larvae is 200g yeast powder, 1000g corn flour, 200g sucrose, 5g sodium benzoate, 1000g pumpkin, 8 ml concentrated hydrochloric acid, 1000 ml water, and 200 g roll paper. The formula of artificial feed for adults is yeast powder: sucrose (w: w) = 1: 1.

2.2. Main equipment

Intelligent artificial climate incubator (ZRX258D, Hangzhou Qianjiang Instrument and Equipment Co., Ltd.), ultra-pure water machine FST-111-TH100 (Thermo), 1/10,000 analytical balance (GR-300, Iand), pipettes with various ranges (Research plus adjustable range pipettes, Ebend China Co., Ltd.), ultraviolet-visible spectrophotometer (UV-vis spectrophotometer). Unico (Shanghai) Instrument Co., Ltd.), Sartorius aquarium Mini Plus pure water ultrapure water machine (H_2O-MA-UV-T).

2.3. Main reagent

CUE (purity 98%, Jiangxi Yixin Perfume Co., Ltd., China), commercial attractant (General Huang, the effective component is pyrimethamine, purity 98%, China Academy of Agricultural Sciences, Beijing, China), MWCNTs-COOH (length 10-20 μm, particle size 30-50 nm, purity 95%, carboxyl content 2.0 wt%). Acetonitrile (AR, 99%), Methylene-Bis-Acrylamide (purity 99%), Poly (ethylene glycol) (PEG 1000, purity 99%), N, N, N', N' -tetramethylethylenediamine (TMEDA, purity 99%) and ammonium persulfate (AR, purity 99%) were all purchased from China Hainan Qingfeng Biotechnology Co., Ltd.

2.4. Experimental methods

2.4.1. Temperature setting

The LCST of temperature-sensitive attractants is approximately 32°C. When the temperature is higher than the LCST of temperature-sensitive attractants, a substantial quantity of attractants will be released (Wang, 2017). Combined with the field temperature, three temperature conditions of 25°C, 33°C and 37°C were set with the help of an artificial climate incubator, while maintaining the humidity at 70±5%.

2.4.2. Setting of trapping time

Chen et al. (2011) discovered that the optimal time for trapping *Z. cucurbitae* Coquillett with CUE is during the morning and noon, and the dosage of CUE in the field was generally about 1 g, which had a good effect. Keiser et al. (1976) found in the experiment that when 0.5 ml of CUE was placed in a glass trap, 1697 *Ceratitis capitata* Wiedemann were trapped within 3 h. Keiser et al. (1976) and Chen et al. (2011) supported these findings and determined that trapping occurred between 9:00 a.m. and 12:00 a.m. with an initial CUE dosage of 0.5 ml.

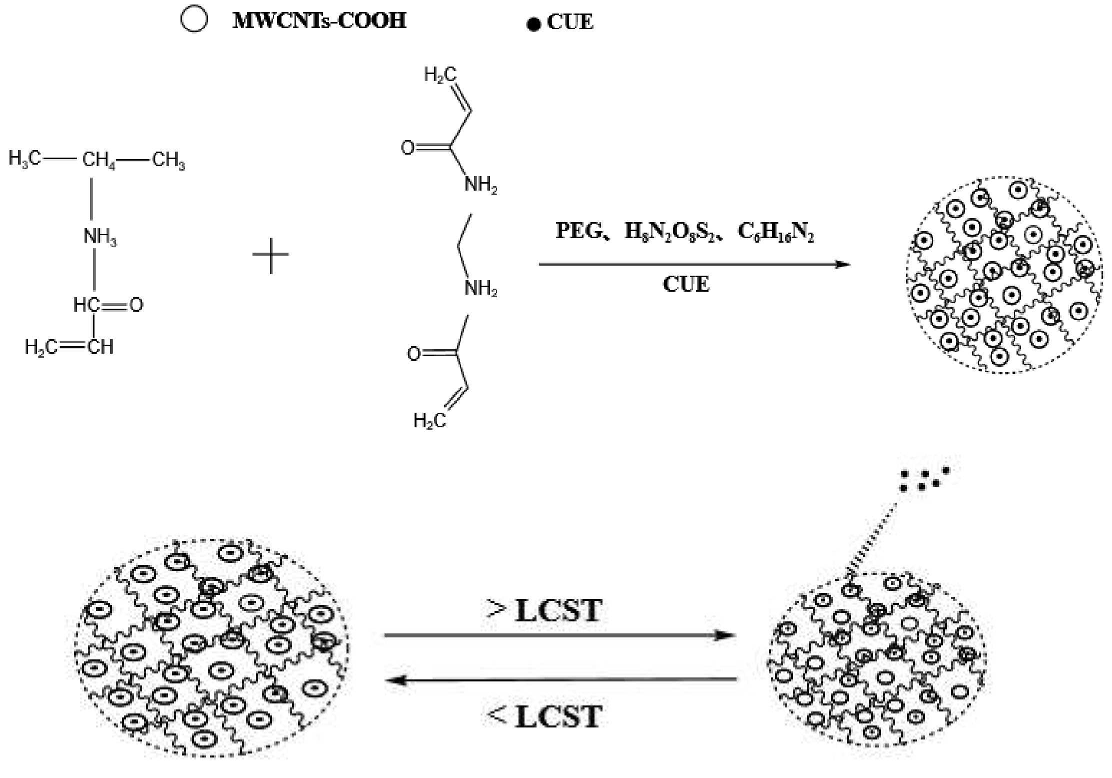
2.5. Evaluation of the trapping effect of CUE on *Z. cucurbitae* Coquillett

To determine the optimal age and dosage of CUE for trapping *Z. cucurbitae* Coquillett under different temperature backgrounds, the cages containing *Z. cucurbitae* Coquillett of 5-day, 10-day, 20-day, and 30-day-old (each cage contains 50 female and 50 male adults) are put into the artificial climatic incubator that had been set up (25°C, 33°C and 37°C, and 70±5% humidity). Then, the traps with different doses of CUE (0.1 ml, 0.5 ml, 1.0 ml, and 2.0 ml) are put into the cage for 3 h, and the treatment without CUE is taken as the blank control (CK_1), with five replicates in each treatment and one replicate for each cage. At the end of the experiment, the number of *Z. cucurbitae* Coquillett trapped in each group was counted and recorded.

2.6. Preparation and evaluation of temperature-sensitive attractant

2.6.1. Preparation of temperature-sensitive attractant

Referring to the method of Pan et al. (2021) and improved, 0.5 ml of CUE was added to 10 ml 60% acetonitrile-water (v/v) solution and mixed evenly, 1.000 g of PEG 1000 was added for ultrasonic treatment for 10 min, then 0.001 g of MWCNTs-COOH were added for ultrasonic dispersion for 30 min, and then sealed for 12 h., Then 1.000 g of NIPAM, 0.02 g of Methylene-Bis-Acrylamide and 50 μL of TEMED were added in turn, stirred and dissolved, and nitrogen was introduced for 30 min to remove oxygen in the system. Finally, 0.02 g of ammonium persulfate was added and nitrogen was continuously introduced until it was completely dissolved, sealed, and reacted at room temperature for 12 h to complete the preparation. The schematic diagram of the synthesis and release of temperature-sensitive attractants



can be found in Fig. 1.

Fig. 1. Schematic diagram of synthesis and sustained-release principle of temperature-sensitive attractant.

2.6.2. Plotting of standard curve of CUE solution

Preparation of CUE stock solution: Accurately weigh 1.000 g CUE pour it into a 100 ml volumetric flask, and use 60% acetonitrile-water (v/v) solution to make the volume constant to 100 ml to prepare 10 mg/ml CUE stock solution.

Preparation of standard solution of CUE: 60% acetonitrile solution was used to dilute the above solution into CUE standard solutions at concentrations of 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml and 7 mg/ml. With 60% acetonitrile as the reference solution, the absorbance of the standard solution of CUE was measured with an ultraviolet-visible spectrophotometer at the wavelength of 270 nm, and the standard curve was drawn with the concentration of the standard solution as the abscissa and absorbance as the ordinate.

2.6.3. The influence of crosslinker content on temperature-sensitive attractants

The content of the cross-linking agent will directly affect the structure and strength of hydrogel. In order to investigate the effect of the amount of cross-linking agent on the performance of temperature-sensitive attractants, the method in step 2.6.1 was used to prepare temperature-sensitive entrapment with different cross-linking agent contents according to Table 1. Then 2.0 g of the prepared temperature-sensitive attractants were immersed in 30 ml 60% acetonitrile solution and placed at 35°C for 3 h. During this period, 1 ml was sampled every 30 min, and 1 ml of the original solution was added to keep the total volume of the solution unchanged. The absorbance of the sample solution was detected, and the concentration of CUE in the solution was calculated by substituting the formula obtained in step 2.6.2.

Table 1

Dosage of crosslinking agent.

Name	Treatment / Reagent	1	2	3	4	5
Thermosensitive monomer (g)	NIPAM	1.0	1.0	1.0	1.0	1.0
Crosslinking agent(g)	Methylene-Bis-Acrylamide	0.02	0.03	0.04	0.05	0.06
Porogen (g)	PEG 10000	1.0	1.0	1.0	1.0	1.0
Accelerating agent (μL)	TMEDA	50	50	50	50	50
Initiator (g)	Ammonium persulphate	0.02	0.02	0.02	0.02	0.02
Solvent (ml)	60% acetonitrile aqueous solution	10	10	10	10	10

2.6.4. Effect of Porogenic Content on Temperature Sensitivity of Temperature Sensitive Attractants

The content of the pore-forming agent directly affects the rate of slow release of the temperature-sensitive attractant. In order to explore the influence of the content of the pore-forming agent on the performance of the temperature-sensitive attractant, the method in step 2.6.1 was used to prepare a temperature-sensitive attractant with different content of pore-forming agent according to Table 2, and then 2.0 g of the prepared temperature-sensitive elicitor was immersed in 30 ml of 60% acetonitrile solution and then placed at 35°C for 3 h. During this period, 1 ml of the samples was taken at intervals of 30 min, and 1 ml of the original solution was replenished to keep the total volume of the solution unchanged. Unchanged, the absorbance of the sample solution was detected and substituted into the formula obtained in step 2.6.2 to calculate the concentration of CUE in the solution.

Table 2

Dosage of pore-forming agent.

Name	Treatment / Reagent	1	2	3	4	5
Thermosensitive monomer (g)	NIPAM	1.0	1.0	1.0	1.0	1.0
Crosslinking agent(g)	Methylene-Bis-Acrylamide	0.02	0.02	0.02	0.02	0.02
Porogen (g)	PEG 10000	0	0.5	1.0	1.5	2.0
Accelerating agent (μL)	TMEDA	50	50	50	50	50
Initiator (g)	Ammonium persulphate	0.02	0.02	0.02	0.02	0.02
Solvent (ml)	60% acetonitrile aqueous solution	10	10	10	10	10

2.7. Morphology characterization of temperature-sensitive attractants

2.7.1. Scanning electron microscope

The prepared PNIPAM hydrogel, PNIPAM porous hydrogel, and PNIPAM@ MWCNTs-COOH@CUE temperature-sensitive inducer were taken as 2 g each, freeze-dried, and an appropriate amount of the solid was sprayed with gold to treat the samples before observing and analyzing the microscopic morphology of the samples using a scanning electron microscope.

2.8. Performance test of temperature-sensitive attractant

2.8.1. Temperature Response Concentration Release Experiment

The speed of drug release in the temperature-sensitive hydrogel can reflect the temperature-sensitive performance of the hydrogel. To evaluate the performance of the temperature-sensitive attractant, multiple portions of the temperature-sensitive attractant prepared in step 2.6.1 (2.0 g each) were put in a centrifuge tube filled with 30 ml 60% acetonitrile solution, and then the centrifuge tubes were placed at 25℃, 30℃, 35℃, 40℃, and 45℃ for 3 h respectively. During this period, 1 ml was sampled every 30 min, and 1 ml of the original solution was added to keep the total volume of the solution unchanged. The absorbance of the sample solution was detected, and then the absorbance value was substituted into the formula obtained in step 2.6.2 to calculate the concentration of CUE in the solution.

2.8.2. Determination of entrapment efficiency and drug loading

As important indicators of key quality attributes of nano-drugs, encapsulation efficiency, and drug loading are closely related to the formulation and preparation technology of nano-drugs, which reflect the degree of drug encapsulation by carriers (Zhang et al., 2022). In order to determine the entrapment efficiency and drug loading of the temperature-sensitive attractant, three samples of 2.0 g temperature-sensitive attractant prepared in step 2.2.1 were weighed and dissolved by 30 ml 60% acetonitrile solution for 30 min, and the water temperature was set at 35℃ during ultrasonic treatment. Centrifuge the supernatant after ultrasonic treatment, measure the absorbance of the solution with the spectrophotometer, and substitute it into the formula obtained in step 2.6.2 to calculate the content of CUE in the solution.

Referring to Singh et al. (2022) and Lim et al. (2017), the encapsulation efficiency (EE) and loading content (LC) of hydrogels were calculated according to the following formulas (1) and (2).

$$EE\ (\%) = M_1/M_2*100\%\ (1)$$

$$LC\ (\%) = M_1/M_3*100\%\ (2)$$

M_1: Total mass of CUE carried in temperature-sensitive attractant; M_2: Initial dosage of CUE; M_3: Total mass of temperature-sensitive attractant.

2.9. The effect of temperature-sensitive attractant on trapping *Z. cucurbitae* Coquillett indoors

2.9.1. Evaluation of the trapping effect of temperature-sensitive attractants on *Z. cucurbitae* Coquillett under different temperature backgrounds

Based on the results of previous experiments, the 20-day-old *Z. cucurbitae* Coquillett is selected as the source of the tested insects. In order to compare the trapping effects of temperature-sensitive attractants, CUE, and commercial attractants, the same dosage (0.1 ml) of these three attractants was put in multiple traps, and the traps were placed in cages containing *Z. cucurbitae* Coquillett (each cage contained 50 female and 50 male adults, and one cage was repeated), and then the cages were placed in different artificial climate incubators (the temperature was 25℃, 33℃ and 37℃, humidity 70±5%). After the experiment, the number of f *Z. cucurbitae* Coquillett trapped in each group was counted and recorded.

2.9.2. Effect of the dosage of temperature-sensitive attractant on trapping *Z. cucurbitae* Coquillett in different temperature backgrounds

In the previous experiment, we found that the dosage of CUE would affect the effect of trapping *Z. cucurbitae* Coquillett. Therefore, in order to evaluate the influence of the dosage of temperature-sensitive attractant on the trapping of *Z. cucurbitae* Coquillett, cages containing *Z. cucurbitae* Coquillett (each cage contains 50 20-day-old females and males, and one cage was repeated) were placed in different artificial climate incubators (temperature 25℃ and 33℃, humidity 70±5%), and then different doses of temperature-sensitive attractant (0 g, 1.0 g, 2.0 g, and 3.0 g) were loaded. After the experiment, the number of *Z. cucurbitae* Coquillett trapped in each group was counted and recorded.

2.9.3. High-temperature resistance limit test of attractant

This study was conducted in Sanya, Hainan, China, which is located in the tropical region, and it is known that the maximum surface temperature in this region can reach 45°C in summer with reference to the local historical temperature records. Considering the use conditions of field attractants and the characteristics of temperature-sensitive attractants, 60°C was chosen as the temperature for the high-temperature test of attractants. The temperature-sensitive attractants, CUE, and commercial attractants were placed in an oven at 60°C for 7 d. On the 1st, 3rd, 5th, and 7th day of treatment, respectively, 0.1 ml of the above attractant was added to the trap, and then the trap was placed in different cages (each cage was filled with 50 20-day-old female and male adults, which was a repetition). finally, the cages were cultured in different artificial climates. After the experiment, the number of *Z. cucurbitae* Coquillett trapped in each group was counted and recorded.

3. Results and analysis

3.1.Evaluation of the trapping effect of CUE on *Z. cucurbitae* Coquillett

3.1.1. Study on the optimum day age of *Z. cucurbitae* Coquillett trapped by CUE under different temperature background

CUE was used to trap 5, 10, 20, and 30-day-old *Z. cucurbitae* Coquillett under different temperature backgrounds. The results show that CUE was effective in trapping *Z. cucurbitae* Coquillett of different ages at different temperatures. At 25°C and 33°C, the number of 20-day-old and 30-day-old *Z. cucurbitae* Coquillett trapped by CUE was the largest, followed by 10-day-old and 5-day-old (Fig. 2). When the ambient temperature was 37 °C, the total number of *Z. cucurbitae* Coquillett trapped by CUE was significantly lower than that at 25 °C and 33 °C, which may be attributed to the fact that *Z. cucurbitae* Coquillett gathered on wet cotton to escape from the high temperature when the ambient temperature was elevated, resulting in a decrease in the number of *Z. cucurbitae* Coquillett trapped by CUE.

In addition, it was found that at 37°C, the number of 30-day-old *Z. cucurbitae*

Coquillett trapped by CUE was significantly higher than that of other days, which may be because the 30-day-old *Z. cucurbitae* Coquillett had higher tolerance to the high-temperature environment than other days, which led to their more activity in the high-temperature environment.

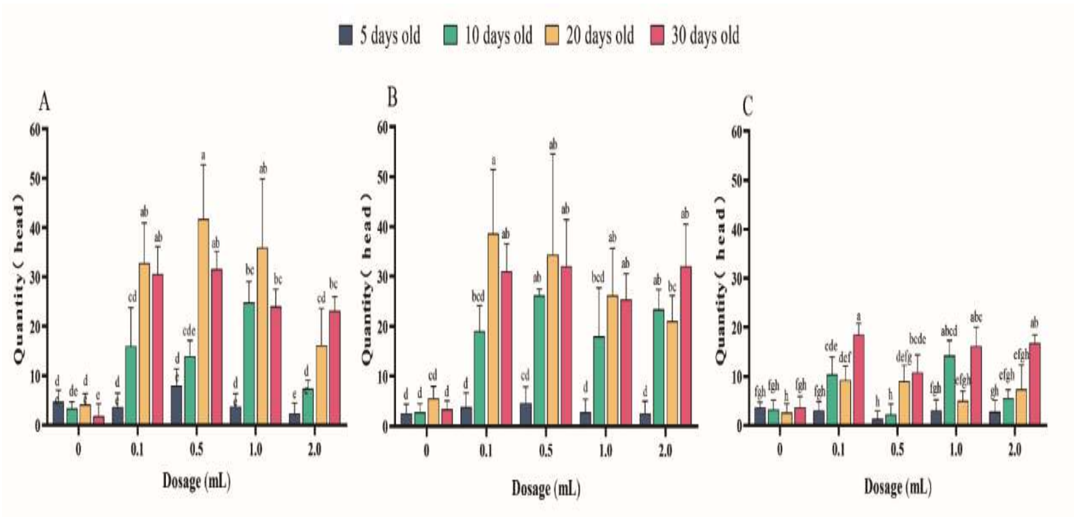


Fig. 2. The number of *Z. cucurbitae* Coquillett of different ages trapped by CUE at different temperatures. (A) 25°C, (B) 33°C, (C) 37°C. The value of the bar chart is mean ± standard error, and the lowercase letters above the bar chart indicate significant differences (P<0.05), as shown in Fig. 4 below.

The results of one-way ANOVA and Tukey's multiple comparisons show that the number of male *Z. cucurbitae* Coquillett trapped by CUE was significantly higher than that of females at 25°C and 33°C (Fig. 3), indicating that muscovite mainly attracted male *Z. cucurbitae* Coquillett. At 37°C, there is no significant difference between them, which may be because, at the high temperature, *Z. cucurbitae* Coquillett gathers in wet cotton to avoid the high temperature, which leads to a small number of *Z. cucurbitae* Coquillett trapped by CUE.

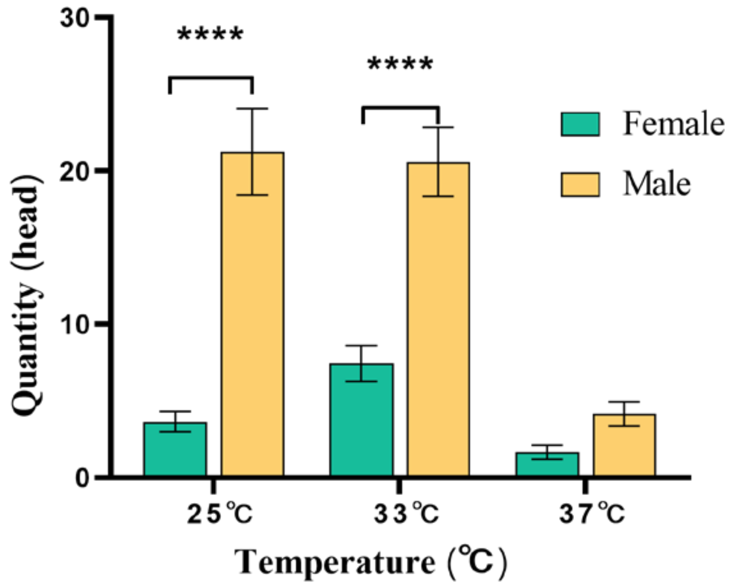


Fig. 3. Number of females and males of *Z. cucurbitae* Coquillett trapped by CUE at different temperatures. Note: the value of the bar chart is mean ± standard error, and the "*", "* *", "* * *" and "* * * *" at the top of the bar chart indicate the significant differences (P<0.05, P<0.01, P<0.001, P < 0.0001, respectively).

3.1.2. The influence of the dosage of CUE on the trapping of *Z. cucurbitae* Coquillett in different temperature backgrounds

Z. cucurbitae Coquillett were trapped at 25°C, 33°C, and 37°C with different doses of CUE. The results show that at 25°C and 33°C, the dosage of CUE was 0.1 ml and 0.5 ml, followed by 1 ml and 2 ml (Fig. 4). It shows that the trapping effect of CUE on *Z. cucurbitae* Coquillett was affected by the dosage of CUE, and its trapping effect would not always increase with the increase of its dosage. The possible reason is that the high concentration of CUE inhibits the sensitivity of male *Z. cucurbitae* Coquillett to CUE, which leads to the decrease of the number of *Z. cucurbitae* Coquillett trapped by CUE when the dosage of CUE is increased to 1 ml and 2 ml.

Under the same dosage of CUE, the number of *Z. cucurbitae* Coquillett trapped by CUE at 25°C and 33°C was significantly higher than that at 37°C, indicating that the environmental temperature at 25°C and 33°C was more favorable for CUE to trap *Z. cucurbitae* Coquillett. The possible reason is that when the environmental temperature was 25-33°C, the physiological activities such as feeding and mating were more active. When the ambient temperature reaches 37°C, the *Z. cucurbitae* Coquillett gathers on wet cotton to avoid the high temperature, which leads to a decrease in the number of *Z. cucurbitae* Coquillett trapped by CUE.

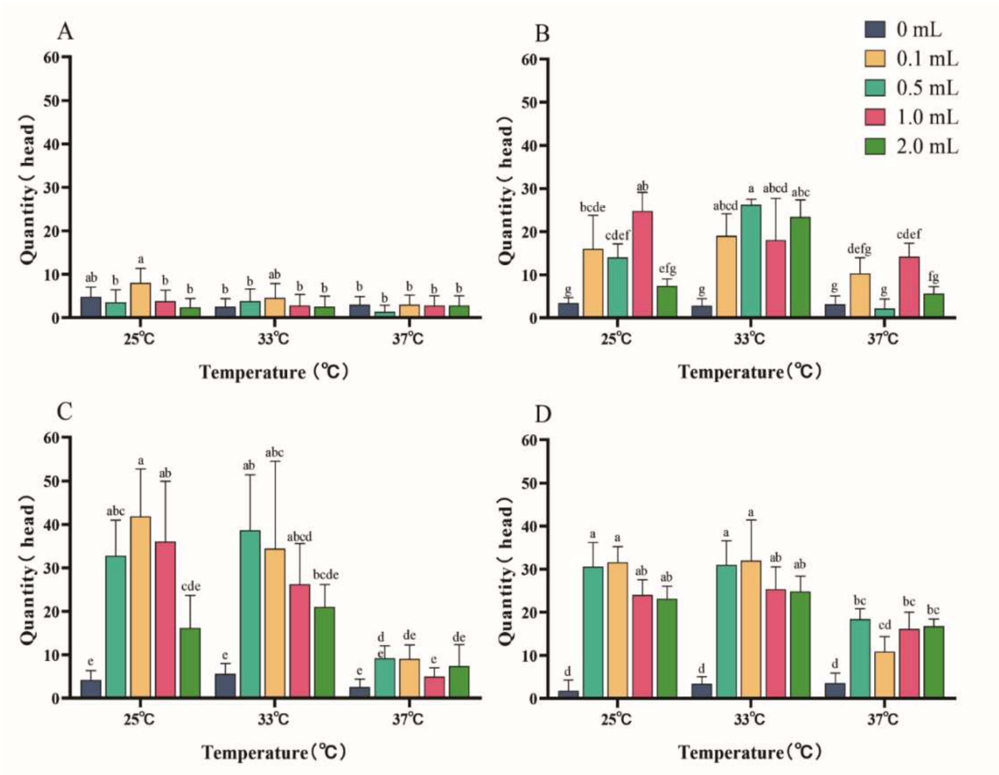


Fig. 4. The number of *Z. cucurbitae* Coquillett trapped by different dosages of CUE. (A) 5 days old, (B) 10 days old, (C) 20 days old, (D) 30 days old *Z. cucurbitae* Coquillett.

3.2. Preparation and evaluation of temperature-sensitive attractant

3.2.1. Drawing of standard curve of CUE

By measuring the absorbance of the standard solution of CUE, the standard curve of absorbance versus CUE concentration was drawn with the concentration of CUE as abscissa and absorbance as ordinate. The results show that there is a linear correlation between the concentration and absorbance of CUE, and the correlation coefficient R^2=0.9926 (Fig. 5), indicating that there is good linearity between the experimental data and the fitting function.

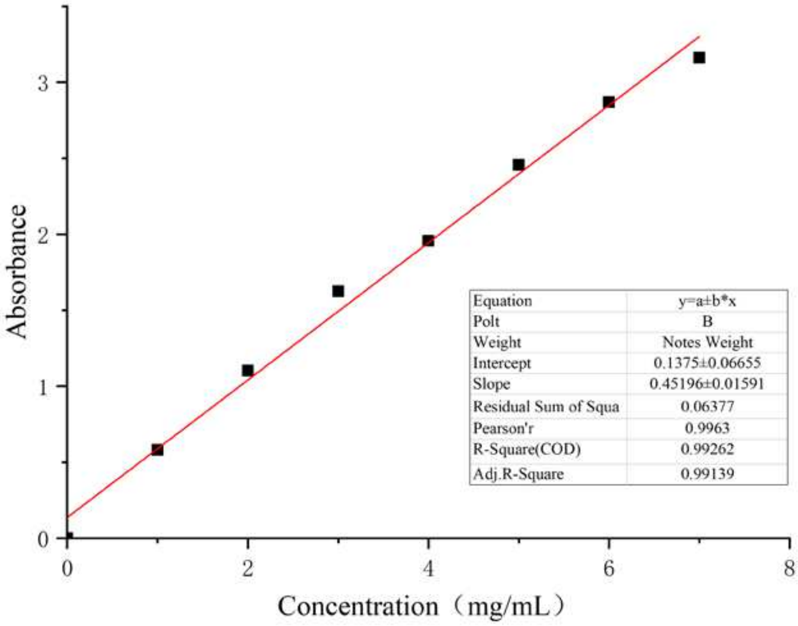


Fig. 5. Standard curve of absorbance versus concentration of CUE.

3.2.2. Effect of crosslinking agent content on temperature sensitivity of temperature-sensitive inducer

The influence of the amount of cross-linking agent on the temperature-sensitive attractant is shown in Fig. 6. The results show that when the amount of cross-linking agent is 0.06 g and 0.03 g, the concentration of CUE in the solution increases within the first 1.5 h, while the release rate of CUE obviously slows down in the process of 1.5-3.0 h. It may be because the CUE in the temperature-sensitive attractant has been released into the solution in the first 1.5 h, which leads to the slow increase of the concentration of CUE in the solution after 1.5 h of the experiment.

When the dosage of the cross-linking agent is 0.05 g and 0.02 g, the release rate of the drug in the temperature-sensitive attractant is relatively gentle, and the concentration of CUE in the solution at 3.0h was only half of that at 0.06g and 0.03g. This indicates that at least half of the remaining CUE in the temperature-sensitive attractant has not been released into the solution. The reason for the change in the release rate of CUE in temperature-sensitive attractants may be that the amount of different crosslinking agents affects the structure of the gel (such as the number of crosslinking points in polymer segments), which leads to the change in the temperature sensitivity of the hydrogel.

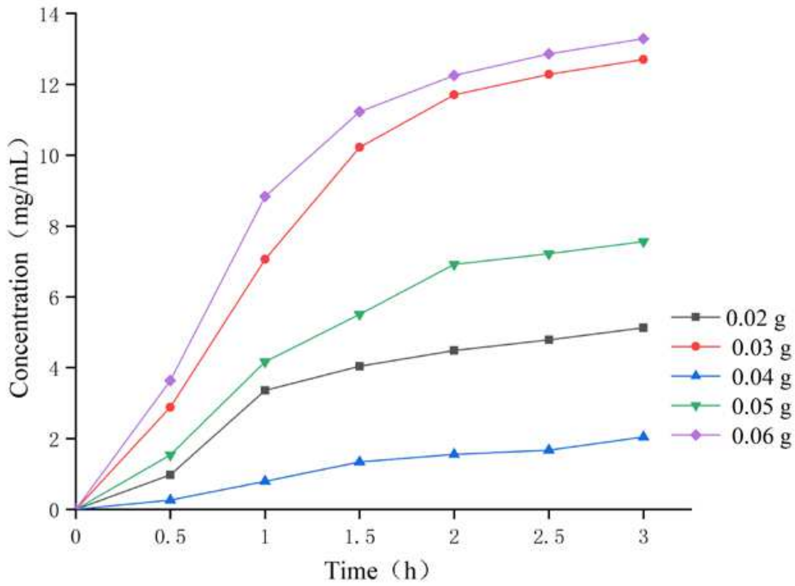


Fig. 6. Effect of dosage of cross-linking agent on temperature sensitivity of temperature-sensitive inducer.

3.2.3. Effect of porogen content on hydrogel temperature sensitivity

The effect of the amount of pore-forming agent on the temperature-sensitive attractant is shown in Fig. 7. The results show that the final concentration of CUE in the solution is inversely proportional to the content of the pore-forming agent. It may be because the pore-forming agent will form many small holes in the gel, which leads to the temperature-sensitive attractant with the less pore-forming agent carrying more CUE per unit volume. In the first 1 hour of the experiment, the release rate and amount of CUE in the temperature-sensitive attractant without a pore-forming agent were higher; After 1 h, the release rate of CUE in the temperature-sensitive attractant without a pore-forming agent was obviously slower than that in other treatments. This is because the surface of the temperature-sensitive sustained-release agent without a pore-forming agent collapses faster than the inside of it, and a dense epidermis layer is formed on its surface, which leads to the inability to release the CUE inside the gel, resulting in a small change in the concentration of CUE in the solution after 1 h. When the dosage of the pore-forming agent is 0.5 g and 1.0 g, the release rate and amount of temperature-sensitive attractant are higher than 1.5 g and 2.0 g, indicating that the temperature-sensitive attractant prepared with the dosage of these two pore-forming agents is more conducive to its slow-release performance.

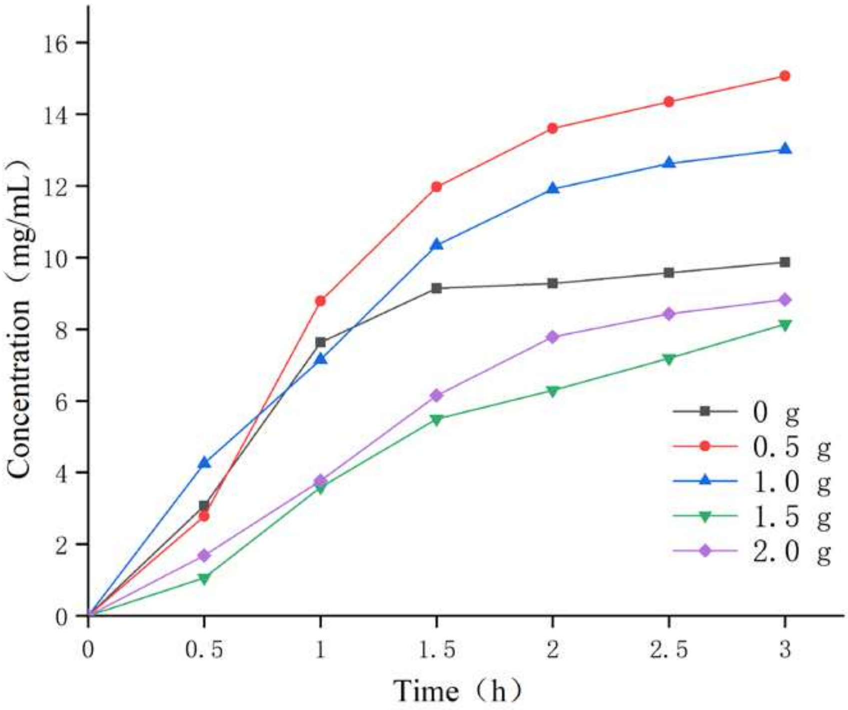


Fig. 7. Effect of the dosage of pore-forming agent on the temperature sensitivity of temperature-sensitive attractant.

3.2.4. Morphology characterization of temperature-sensitive attractants

The scanning electron microscope (SEM) image of the hydrogel sample is shown in Fig. 8. There are only a few pores on the surface of the gel without a pore-forming agent (Fig. 8A). In contrast when the pore-forming agent is added to the hydrogel, it can be clearly seen that a large number of pores are generated on the surface of the gel (Fig. 8B). The existence of these pores will be beneficial to the release of CUE in the temperature-sensitive attractant. By observing the prepared PNIPAM@ MWCNTs-COOH@CUE temperature-sensitive attractant at the scale of 100 μm, it can be clearly seen that there are many holes in the network structure of the gel (Fig. 8C), which not only provide sufficient channels for the release of CUE but also provide large-area attachment sites for carbon nanotubes, providing them with the ability to carry more CUE.

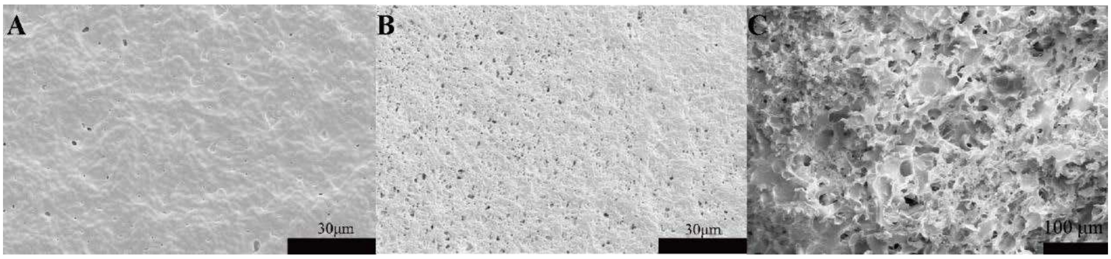


Fig. 8. SEM image of hydrogel. (A) PNIPAM hydrogel, (B) PNIPAM porous hydrogel, (C) PNIPAM@ MWCNTs-COOH@CUE @ CUE temperature-sensitive attractant.

3.3. Performance test of temperature-sensitive attractant

3.3.1. Temperature response release experiment of temperature-sensitive trap

The experimental results of temperature-responsive release of temperature-sensitive attractants are shown in Fig. 9. The results show that the release rate of temperature-sensitive attractants is positively correlated with temperature in the range of 25- 45°C. When the temperature is 25°C, the drug release rate of the temperature-sensitive attractant is slow. However, when the temperature rises to 35°C and 45°C, the release rate of the drug in the temperature-sensitive attractant increases obviously in unit time, and the final release concentration of the drug is also higher than 25°C. These findings suggest that the temperature-responsive attractant exhibits favorable responses to changes in temperature.

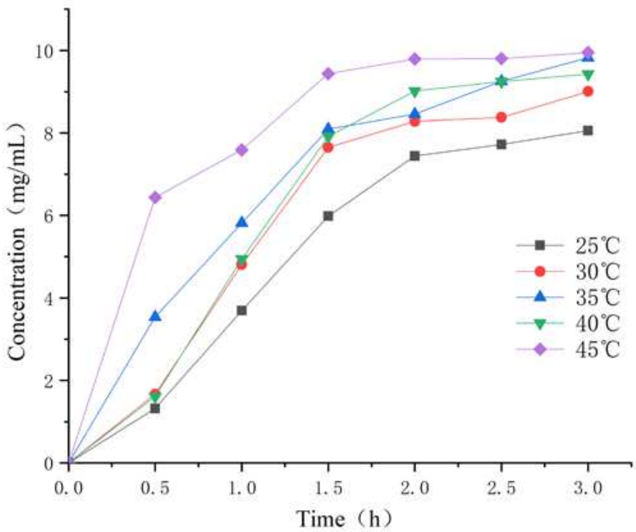


Fig. 9. Temperature response release of temperature-sensitive attractant.

3.3.2. Determination of entrapment efficiency and drug loading

The entrapment efficiency of the temperature-sensitive attractant is a crucial indicator of the carrier's performance. In this study, the entrapment efficiency of the temperature-sensitive attractant prepared is 73.34-75.53%, and the drug loading is 3.36-3.46% (Table 3). The results demonstrate that the attractant has good drug-loading performance.

Table 3

Entrapment efficiency and drug loading of temperature-sensitive attractants

sample	Absorbance	concentration （mg/ml）	Entrapment efficiency（%）	Drug loading（%）
1	1.186±0.01	2.32±0.01	75.53±0.01	3.46±0.02
2	1.174±0.01	2.29±0.02	74.83±0.01	3.43±0.03
3	1.182±0.01	2.31±0.03	73.34±0.01	3.36±0.04

3.4. Indoor effect determination

3.4.1. Evaluation of the effect of temperature-sensitive attractant on trapping *Z. cucurbitae* Coquillett

The results of trapping *Z. cucurbitae* Coquillett indoors with a temperature-sensitive attractant are shown in Fig. 10. Under the temperature background of 25℃ and 33℃, there is no significant difference between the number of *Z. cucurbitae* Coquillett trapped by temperature-sensitive attractant and the number of *Z. cucurbitae* Coquillett trapped by using CUE alone. This indicates that the efficacy of trapping *Z. cucurbitae* Coquillett using CUE remains unimpaired during the preparation process of the temperature-sensitive attractant. At 37℃, a greater number of *Z. cucurbitae* Coquillett are trapped using the temperature-sensitive attractant than with CUE alone. The possible reason is that the temperature at this time is above the LCST of the temperature-sensitive attractant, which led to the rapid volatilization of the pheromone in the temperature-sensitive attractant, thus increasing the effect of trapping *Z. cucurbitae* Coquillett by the temperature-sensitive attractant.

There is no significant difference in the number of *Z. cucurbitae* Coquillett trapped by commercial attractants and temperature-sensitive attractants under the three temperature backgrounds of 25°C, 33°C, and 37°C, indicating that the prepared temperature-sensitive attractants had a good application potential. At 25°C, the number of *Z. cucurbitae* Coquillett trapped by temperature-sensitive attractants is less than that of the commercial attractants. When the temperature is increased to 33°C and 37°C, the number of *Z. cucurbitae* Coquillett trapped by temperature-sensitive attractants is more than that of commercial attractants. This is because when the ambient temperature is 25°C, the LCST of the temperature-sensitive attractant is not reached, and the release rate of CUE in the attractant is slow, which leads to the number of trapped *Z. cucurbitae* Coquillett being less than that of commercial attractants. When the temperature reaches 33°C and 37°C, which is higher than the LCST of the temperature-sensitive attractant, a large quantity of CUE is released in a short time, which increases the effectiveness of the lure in trapping *Z. cucurbitae* Coquillett.

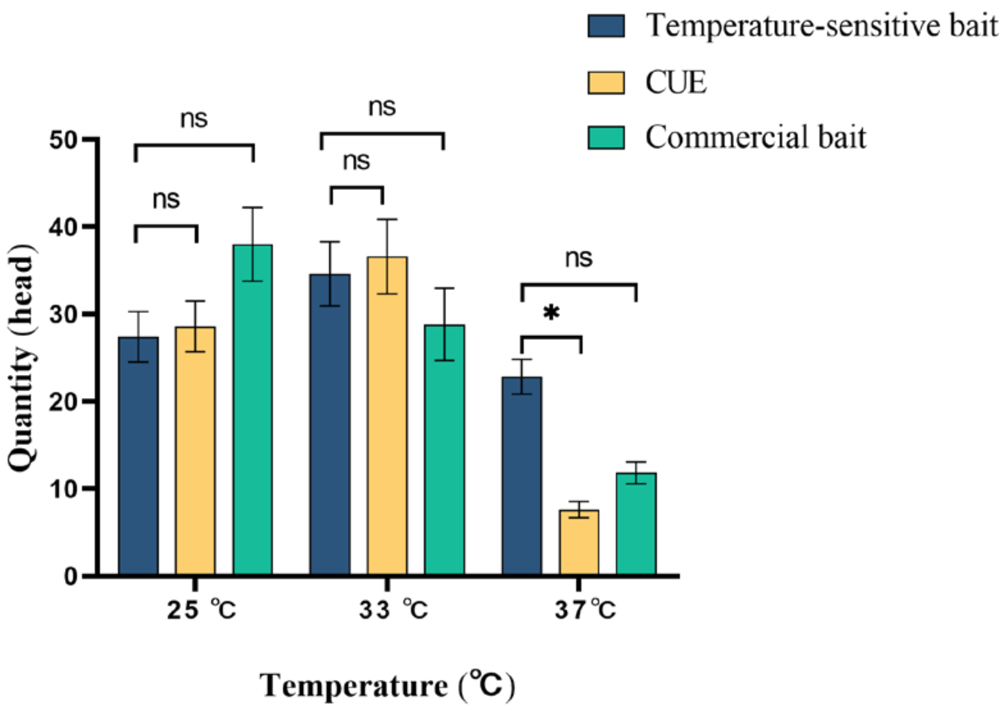


Fig. 10. The number of *Z. cucurbitae* Coquillett trapped by three attractants at different temperatures.

3.4.2. Effect of the dosage of temperature-sensitive attractant on trapping *Z. cucurbitae* Coquillett

The number of *Z. cucurbitae* Coquillett trapped by different dosages of temperature-sensitive attractants is shown in Fig. 11. The results show that at the same temperature, the number of trapped *Z. cucurbitae* Coquillett trapped by 2 g of temperature-sensitive attractants is more than that of other treatments. It shows that the trapping effect was the best when the dosage of temperature-sensitive attractant was 2 g, and it also shows that the trapping effect of CUE would not always increase with the increase of its dosage, which was consistent with the experimental results in 3.1.2. The possible reason is that when the dosage of the temperature-sensitive attractant is 2 g, the release rate of CUE in the attractant reaches an optimal level, resulting in the highest number of *Z. cucurbitae* Coquillett trapped.

When the dosage of temperature-sensitive attractant is the same, the number of *Z. cucurbitae* Coquillett trapped at 33°C is more than 25°C. This is because when the temperature is 25°C, the ambient temperature does not reach the LCST of the temperature-sensitive attractant, and only a small amount of CUE is released. When the ambient temperature reaches 33°C, the structure of the temperature-sensitive attractant changes phase, and a large amount of CUE in the temperature-sensitive attractant is released into the environment, thereby attracting more *Z. cucurbitae* Coquillett. The results show that the temperature-sensitive attractant can respond to the stimulation of external temperature and thus change the release rate of the attractant.

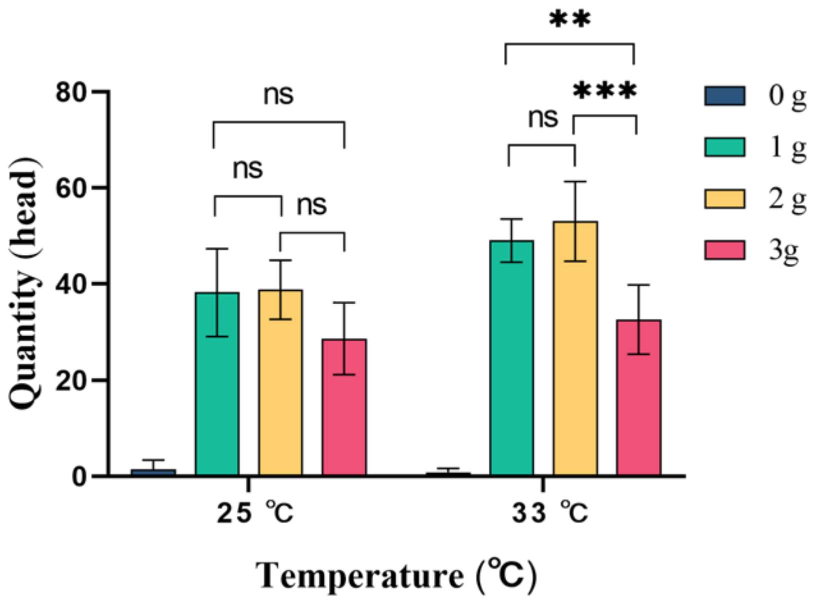


Fig. 11. The number of *Z. cucurbitae* Coquillett trapped by different dosages of temperature-sensitive attractants.

3.4.3. High-temperature resistance test

The high-temperature resistance test results of temperature-sensitive attractants are shown in Fig. 12. The results show that the number of *Z. cucurbitae* Coquillett trapped by commercial attractants, temperature-sensitive attractants, and CUE is negatively correlated with the treatment time, which implies that high temperature will reduce the trapping effect of these three attractants on *Z. cucurbitae* Coquillett. Five days before high-temperature treatment, the number of *Z. cucurbitae* Coquillett trapped by commercial attractants and CUE is more than that of temperature-sensitive attractants. By the 5th day, the number of *Z. cucurbitae* Coquillett trapped by the three attractants was close to 40. On the 7th day, the number of *Z. cucurbitae* Coquillett trapped by commercial attractants and CUE decreased to less than 40, while the number of *Z. cucurbitae* Coquillett trapped by temperature-sensitive attractants remained above 40. This may be due to high temperatures accelerating the volatilization of these attractants. Although a large amount of solution in the temperature-sensitive attractant volatilizes, due to the existence of carbon nanotubes, a large amount of CUE is adsorbed in the inner space of carbon nanotubes, which leads to the slow volatilization rate of CUE in the temperature-sensitive attractant, thus prolonging its trapping effect.

The results of one-way ANOVA and Tukey's multiple comparisons show that there is a significant difference in the number of *Z. cucurbitae* Coquillett trapped by commercial attractants on the first and seventh day ($P < 0.01$), while there is no significant difference in the number of *Z. cucurbitae* Coquillett trapped by CUE and temperature-sensitive attractants. This suggests that compared with commercial attractants, CUE has better high-temperature resistance. Compared with CUE, the number of *Z. cucurbitae* Coquillett trapped by the temperature-sensitive attractant is more than 40, which shows that the high-temperature treatment for seven days has no significant effect on the trapping effect of the temperature-sensitive attractant. This indicates that the temperature-sensitive attractant has excellent high-temperature resistance.

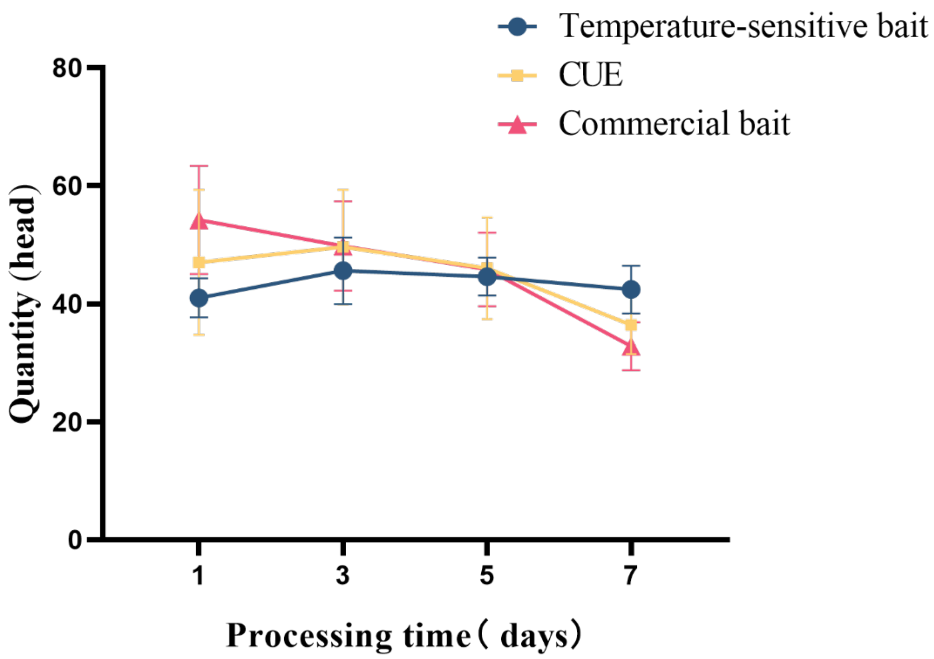


Fig. 12. High-temperature resistance test of three attractants. Note: the value of the line chart is mean ± standard error.

4. Discussion

Sexually mature *Z. cucurbitae* Coquillett is more likely to be attracted by CUE. The results of this study suggest that the attraction of CUE to 10-day-old, 20-day-old, and 30-day-old *Z. cucurbitae* Coquillett is significantly higher than that of 5-day-old *Z. cucurbitae* Coquillett (Fig. 2). This indicates that the trapping ability of CUE to *Z. cucurbitae* Coquillett may be related to the degree of sexual development of *Z. cucurbitae* Coquillett. Combined with the research of Yuan et al. (2005), it was found that *Z. cucurbitae* Coquillett began mating after reaching sexual maturity 9-11 d after emergence, which further confirmed the results of this study. According to the literature, CUE has been documented as an attractant for male *Z. cucurbitae* Coquillett. However, interestingly, our study reveals that about one-sixth of the total number of female *Z. cucurbitae* Coquillett collected in traps (Fig. 3), which is inconsistent with the literature. For this reason, we conducted a validation experiment in which a trap containing 0.5 ml of CUE is placed in a cage containing only female *Z. cucurbitae* Coquillett for 3 h. At the end of the experiment, no female *Z. cucurbitae* Coquillett are collected in the trap, so what are the female *Z. cucurbitae* Coquillett attracted to in this study? Upon reviewing the literature, Shelly et al. (1995) found that the male *Z. cucurbitae* Coquillett that ate the pheromone were more attractive to females than those that did not eat CUE. This suggests that the female *Z. cucurbitae* Coquillett in the trap in this study is attracted by the male who had eaten the CUE.

In addition, it was found in this study that the attraction effect of CUE to *Z. cucurbitae* Coquillett does not always increase with the increase of its use. A significant decrease in the number of *Z. cucurbitae* Coquillett trapped by CUE when the dosage of CUE is increased from 0.5ml to 1 ml and 2 ml, (Fig. 4). Similar results are obtained in the experiment evaluating the trapping effect of temperature-sensitive attractants on *Z. cucurbitae* Coquillett. When the dosage of temperature-sensitive attractants is increased from 1 g and 2 g to 3 g, the number of *Z. cucurbitae* Coquillett trapped by the attractants also decreases (Fig. 11), suggesting that the high dose of CUE might inhibit the sensitivity of male *Z. cucurbitae* Coquillett to it.

PNIPAM hydrogel is a material with good temperature sensitivity. Because of its characteristics of no pollution to the environment, no biotoxicity, and recyclability, PNIPAM hydrogel has good application prospects in the field of pesticide-sustained release (Gheysoori, et al. 2023). For example, Feng et al. (2020) combined starch, alginate, kaolin, and PNIPAAM to prepare a semi-interpenetrating network hydrogel with temperature sensitivity, and the research results showed that semi-interpenetrating network hydrogel beads containing kaolin not only have a slow-release effect before peanut flowering but also can release biocontrol agents rapidly after flowering. Xu et al. (2017) used polydopamine (PDA) microspheres as the photothermal agent, and then encapsulated with PNIPAM hydrogel, and prepared a core-shell nanocomposite. The nanocomposite shows high loading capacity and temperature or near-infrared controlled release properties, which had great potential in the field of drug-controlled release. In this study, the temperature response performance of the temperature-sensitive attractant is tested and the results show that both the release rate and final concentration of CUE are positively correlated with the temperature. Wu et al. (2005) found that the higher the environmental temperature, the faster the drug release and the more the cumulative release. This result is consistent with our research results, indicating that the temperature-sensitive attractant prepared in this study has good temperature sensitivity.

PNIPAM hydrogels can not only respond to changes in external temperature and thus control the release of drugs but also maintain the stability of the drugs loaded. For example, Wang et al. (2021) obtained a temperature-responsive release pesticide formulation by modifying graphene oxide with PNIPAM hydrogel and then loading lambda-cyhalothrin onto the nanocomposite carrier. The pesticide formulation not only could control the release of lambda-cyhalothrin at a specific temperature and prolong its sustained release time but also had good water dispersibility and UV resistance. In this study, the high-temperature resistance of the temperature-sensitive attractant is tested. The results show that, after being treated at 60°C for 7 d, compared with CUE and commercial attractants, the attraction ability of the temperature-sensitive attractant to *Z. cucurbitae* Coquillett is not obviously weakened compared, indicating that the temperature-sensitive attractant also has good stability in the high-temperature environment.

Since the loading of CUE relies on the network structure inside the carbon nanotubes and PNIPAM hydrogel, without any other special conditions. Therefore, other components (such as chemical pesticides) can be mixed into the temperature-sensitive attractant to further enhance the pest control effect of the attractant. In addition, CUE can be replaced with other attractants to make different types of slow-release agents, thus expanding its application scope.

Acknowledgements and Funding

The research was supported by the Project of Sanya Yazhou Bay Science and Technology City (SCKJ-JYRC-2023-14), Open Project of Key Laboratory of Tropical Fruit Tree Biology of Hainan Province (HAAS2023PT0206) and Hainan Major Science and Technology Project (ZDKJ201901).

Author contributions

AQW, SHP, BZ, YYL, SYY, JJJ, BW, QKZ, CCN, MKL, JT, JJC, QXW, XFY, JJLand SHZ participated in the study design and analysis the manuscript.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Arya, V., Srinivasa, N., Tyagi, S., Raju, S.V.S., 2022. A guide to prepare Cue-Lure for *Bactrocera cucurbitae* (Coquillett) management in cucurbits. Indian Entomologist. 3(1): 45-47.

Baydin, A., Tay, F., Fan, J., Manjappa, M., Gao, W.L., Kono, J., 2022. Carbon Nanotube Devices for Quantum Technology. Materials. 15(4): 1535.

Beroza, M., Alexander, B.H., Steiner, L.F., Mitchell, W.C., Miyashita, D.H., 1960. New synthetic lures for the male melon fly. Sci. 131(3406): 1044-1045.

Biswas, M., Hossain, J., Mainali, B., Inskeep, J.R., Gaire, S.K., Cross, D., D. Stringer, L., W. Taylor, P., Rempoulakis, P., 2022. Extended holding period and yeast hydrolysate in pre-release diet increase abundance of mature sterile Queensland fruit fly males in the field. Journal of Pest Sci. 95(1): 291-301.

Chen, X., Feng, C.H., Shen, Y.L., Lin, D.Q., Feng, B., He, Z.Q., Du, Y.J., 2011. Chemical synthesis of cuelure and its attractive activities to *Bactrocera cucurbitae*. China Plant Protection. 31(9): 9-13.

Chen, Y., Ma, C.S., 2010. Effect of global warming on insect: a literature review. Sheng Tai Xue Bao. 30(8): 2159-2172.

Feng, J.C., Dou, J.P., Zhang, Y.Z., Wu, Z.D., Yin, D.X., Wu, W.F., 2020. Thermosensitive hydrogel for encapsulation and controlled release of biocontrol agents to prevent peanut aflatoxin contamination. Polymers. 12(3):547.

Gheysoori, P., Paydayesh, A., Jafari, M., Peidayesh, H., 2023. Thermoresponsive nanocomposite hydrogels based on Gelatin/poly (N–isopropylacrylamide) (PNIPAM) for controlled drug delivery. Eur Poly J. 186: 111846.

Guptaa, A., Regmib, R., 2022. Efficacy of different homemade and commercial baits in monitoring of fruit flies at Maranthana, Pyuthan, Nepal. MJSA. 6(2): 101-109.

Keiser, I., Jacobson, M., Nakagawa S, H. Miyashita, D., J. Harris, E., 1967. Mediterranean fruit fly: attraction of females to acetic acid and acetic anhydride, to two chemical intermediates in the manufacture of Cue-lure and to decaying Hawaiian tephritids. J. Econ. Entomol. 69(4): 517-520.

Kumaran, N., Hayes, R.A., R. Clarke, A., 2014. Cuelure but not zingerone make the sex pheromone of male *Bactrocera tryoni* (Tephritidae: Diptera) more attractive to females. J. Insect Physiol. 68: 36-43.

Li, Mao, Wei, X.M., Li, j., Wei, S.M., Zhang, J.L., Chen, G.H., Zhang, X.M., 2023. Effect of short-term exposure to high temperatures on the reproductive behavior and physiological enzyme activities in the fruit fly *Zeugodacus tau* (Walker). Front. Physiol. 14: 1036397.

Lim, G.P., Ahmad, M.S., 2017. Development of Ca-alginate-chitosan microcapsules for encapsulation and controlled release of imidacloprid to control dengue outbreaks. J Ind Eng Chem. 56: 382-393.

Lu, Y.T., Zeng, K., Fuhrmann, B., Woelk, C., Zhang, K., Groth, T., 2022. Engineering of stable cross-linked multilayers based on thermo-responsive PNIPAM-grafted-chitosan/heparin to tailor their physiochemical properties and biocompatibility. ACS Appl. Mater. Interfaces. 14(26): 29550-29562.

Ma, G., Ma, C.S., 2016. The impacts of extreme high temperature on insect populations under climate. Chinese Bulletin of Life Sciences. 46(5): 556-564.

Majstorović, N., Agarwal, S., 2022. Strong, stretchable, dual-Responsive PNIPAM nanogel

cross-linked UCST-type macrogels for biomedical applications. ACS Appl. Polym. Mater. 4(8): 5996-6005.
Maula, F., Khan, A.A., Ali, A., Inamullah, Younus, M., Israr, M., Rauf, M.A., Ahmad, I., 2022. Evaluation of different traps and lures combinations for monitoring and eco-friendly management of fruit fly (*Bactrocera Spp*) in peach orchards. J. Entomol. 10(1): 105-110.
Pan, S.P., Tao, Q., Wang, Y.T., 2021. Study on the preparations and properties of temperature-responsive injectable hydrogel based on poly (N-isopropylacrylamide). Modern Chemical Research. (22):7-9.
Shelly, T., Epsky, N., Jang, E.B., Reyes-Flores, J., Vargas, R., 2014. Trapping and the detection, control, and regulation of tephritid fruit flies: lures, area-wide programs, and trade implications. Springer.
Shelly, T.E., Villalobos. E.M., 1995. Cue lure and the mating behavior of male melon flies (Diptera: Tephritidae). Fla Entomol. 473-473.
Singh, A., Kar, A.K., Singh, D., Verma, R., Shraogi, N., Zehra, A., Gautam, K., Anbumani, S., Ghosh, D., Patnaik, S., 2022. PH-responsive eco-friendly chitosan modified cenosphere/alginate composite hydrogel beads as carrier for controlled release of Imidacloprid towards sustainable pest control. Chem. Eng. J. 427: 131215.
Wang, Q., 2017. Synthesis and properties of thermo-responsive copolymers based on N-sopropylacrylamide. Shenzhen University.
Wang, Y.L., Song, S.J., Chu, X.H., Feng, W.L., Li, J., Huang, X.R., Zhou, N.L., Shen, J., 2021. A new temperature-responsive controlled-release pesticide formulation - poly (N-isopropylacrylamide) modified graphene oxide as the nanocarrier for lambda-cyhalothrin delivery and their application in pesticide transportation. Colloid Surface A. 612, 125987,
Wu, J.Y., Liu, S.Q., Heng, P.W.S., Yang, Y.Y., 2005. Evaluating proteins release from, and their interactions with, thermosensitive poly (N-isopropylacrylamide) hydrogels. Journal of Controlled Release. 102(2): 361-372.
Xiao, Q.Y., Cui, Y.K., Meng, Y.S., Guo, F., Ruan, X.H., He, G.H., Jiang, X.B., 2022. PNIPAm hydrogel composite membrane for high-throughput adsorption of biological macromolecules. Sep. Purif. (294): 121224.
Xu, X.H., Bai, B., Wang, H.L., Suo, Y.R., 2017. A near-infrared and temperature-responsive pesticide release platform through core–shell polydopamine@ PNIPAm nanocomposites. ACS Appl. Mater. Interfaces. 9(7): 6424-6432.
Yazdani, M., 2022. Developing lines of Queensland fruit flies with different levels of response to a kairomone lure. Insects. 13(8): 666.
Yuan, S.Y., Kong, Q., Li, Z.Y., Xiao, Z.Y., Chen, B., Zhang, D.G., 2005. Study on biology of *Bactrocera* (*Zeugodacus*) *cucurbitae*. Acta Agriculturae Boreali-occidentalis Sinica. 3: 43-45+67.
Zhang, R., Wen, Q.N., Wu, G.Y., Shi, Y., Xu, P., 2022a. Reliability of ultrafiltration centrifugation in the evaluation of entrapment efficiency: A case study of Panax notoginseng saponins chitosan nanoparticles. Chinese Pharm. J. 57(16):1367-1373.
Zhang, X., Zhang, W.Q., Dai, J.Y., Sun, M.Z., Zhao, J., Ji, Y.F., Chen, L., Zeng, F.L., Yang, F.C., Huang, B.L., Dai, L.M., 2022b. Carboxylated carbon nanotubes with high electrocatalytic activity for oxygen evolution in acidic conditions. InfoMat. 4(1): e12273.
Zhang, X.K., Meng, L.J., Lu, Q.H., Fei, Z.F., J. Dyson, P., 2009. Targeted delivery and controlled

release of doxorubicin to cancer cells using modified single wall carbon nanotubes. Biomaterials. 30(30): 6041-6047.

Zhou, S.H., 2016. Studies on the responses of *Bactrocera cucurbitae* to high temperature stress and itsmoelcular basis. Hainan University.