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Research Article

Nematicidal Action of Microencapsulated Essential Oil of Flesh Fingered Citron

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Flesh fingered citron (FFC) essential oil (EO) is susceptible to volatilisation at room temperature. Therefore, its use as a nematicide requires a controlled release. In the present study, we encapsulated FFC EO in β-cyclodextrin by embedding and investigated release from the capsules compared to unembedded EO. We evaluated the structural and thermal properties of the capsules by SEM and TGA. The loading capacity was 32.67%, and the embedding yield was 96.24%, assuming that a core-to-wall quality ratio of 1 : 6 is optimal for the carrier. Using *Caenorhabditis elegans* as a model organism, we explored the toxicity of (1) FFC EO microcapsules (MCs) and (2) four key compounds of the EOs. The MCs enabled sustained release, e.g., 77% mortality after 4 h and 100% within an additional half-hour. The four main compounds in EO can each kill nematodes by reducing antioxidant activity. Since microencapsulation can improve FFC EO stability and prevent product loss due to adverse environments exposed to the air, encapsulating FFC EO in MCs has great potential as a new nematicide.

1. Introduction

Worldwide, nematodes cost as much as $157 billion (USD) per year in economic damage. Severe nematode diseases are frequent and continue to spread, resulting in a continually decreasing crop yield. Due to nematodes' high species diversity and extremely rapid reproduction, farmers have difficulty selecting an appropriate nematode control method. Synthetic pesticides are expensive, and environmental hazards include contaminated soil and water, high persistence, toxicity to humans and other animals, and residues in food. Farmers need new nematode biocontrol measures that are nonsynthetic, cheaper, and more environmentally friendly [1, 2].

In this context, natural products and their derivatives—important sources of novel therapeutics [3]—may be useful. For example, industries including cosmetics, food packaging and processing, and agriculture use plant essential oils (EOs) [4, 5]. EOs can exhibit toxic, repellent, and antifeedant effects on insects and nematodes [6–8]. EO from flesh fingered citron (FFC; from citrus) are generally extracted mechanically [9]. FFC EO has antimicrobial, antifungal, and anthelmintic properties, and functions through various modes of action: (a) antirespiratory (analogously to fumigants), (b) prevent through exposure or ingestion experiments, (c) antireproductive (including reduced fertility and sterilization), (d) antifeedant, or (*e*) a combination thereof. However, the biological activity of EOs may be lost by volatilisation or degradation, and repeated applications are necessary to achieve the effective nematicidal activity [10, 11]. Thus, commercial applications of EOs are limited [12].

Microcapsules (MCs) can address these limitations. By encapsulating an active compound, MCs are a potential platform to accomplish nematicide. Microencapsulation to entrap EOs is one of the most effective methods of applying nematicides, due to "packaging" the active ingredients in the wall material, thereby converting the liquid into a more stable powder [13]. The release of the functional agent occurs by diffusion of the capsule wall and/or MC rupture. Therefore, MCs provide a durable nematicide repellent and lethal toxin [14].

To test the utility of EOs for biocontrol, *Caenorhabditis elegans* nematodes are good model organisms in many fields, such as biomedicine, environmental toxicity, aging, metabolism, obesity, and drug discovery. *C. elegans* are good model organisms because they are easy to grow and inexpensive to

maintain in the laboratory, have a relatively short lifecycle, and are small [15].

In this paper, we characterized MCs that consist of β-cyclodextrin (β-CD)-packaged EO of FFC. We evaluated MC properties by measuring the embedding and release rates. We analyzed MC molecular structure and thermal stability. We quantitated MC nematicidal activity. We also verified that each of the four main compounds in the EO acts as a nematicide. Our results will be useful for minimising how frequently EOs must be applied to retain nematicidal activity.

2. Materials and Methods

2.1. Chemicals, Plant Materials, and C. elegans. β-CD, D-limonene (95%), α-pinene (98%), β-pinene (≥95%), and γ-terpinene (95%) were obtained from Macklin (China). Potassium dichromate ($K_2Cr_2O_7$) and dimethyl sulphoxide (DMSO) were purchased from Sigma (USA). All other chemicals and solvents were of analytical grade. Mature fruits of FFCs (Latin name: *Citrus medica* L. *var Sarcodactylis* (*Noot.*)) were harvested in November 2017, from a local orchard in Jinhua city, Zhejiang province, China. Wild-type *C. elegans* (N2) and food *Escherichia coli* OP50 strains were obtained from the *Caenorhabditis* Genetics Centre.

2.2. EO Preparation and GC–MS Analysis. Mechanical pressing is suitable for extracting easily burnt raw materials such as citrus and lemon because it is easy to maintain the structure and function of EOs. The FFC peels were saturated in 2% (w/v) calcium chloride or calcium hydroxide for 5 h, and then washed with distilled water. The soaked flavedo (50 g) was weighed and turned into fruit juice with 0.25% sodium chloride added to dissolve it. After stratification, the upper oil layer of the mixture was separated and dried over anhydrous sodium sulphate. The resulting EO was kept at 4°C until further analysis. The purification was conducted using molecular distillation (Pope, USA). The extraction rate of the EOs was approximately 0.27%.

A fused silica capillary column (HP-5MS) was used for GC–MS analysis with a Hewlett–Packard 6890 Gas Chromatograph (5% phenyl-methyl siloxane, 60 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA). We determined the percentage of the oil components in the EO by quantitating the peak area. The relative content of each component was calculated by area normalization [16].

2.3. Preparation of Microcapsules and Characterization

2.3.1. Preparation of Microcapsules. Microencapsulation was using the embedding method. A certain amount of β-CD was added to water and dissolved at 80°C to obtain a saturated solution. Adding 10% essential oil of ethanol solution under stirring, preparing a solution of EO: β-CD in different proportions 1 : 5, 1 : 6, and 1 : 7, maintaining 50°C and stirring for 2 h, and then naturally cooling to room temperature, at 4°C for 24 hours. Then suction was filtered with a Buchner funnel and washed.

2.3.2. Percent Yield and LC Value of Microcapsules. The embedding yield of the microcapsules is calculated as a percentage of the total amounts of β-CD and essential oils employed during preparation. The percentage yield of the microcapsules was calculated by using the following formula [17]:

$$\%\text{yield} = \left[\frac{\text{actual amount of microcapsules obtained}}{\text{total (amount of oil + amount of } \beta - \text{CD)}}\right] \times 100. \quad (1)$$

The loading capacity (LC) is defined as the mass percentage of the encapsulated essential oils, which reflects the microcapsule loading ability. Using the thermogravimetric analyzer (TGA) to perform thermal weight test on the blank microcapsules and the essential oil of flesh fingered citrons microcapsules powder respectively, calculates the loading amount of the essential oil in the microcapsules. TGA test conditions are as follows: room temperature ~600°C; heating rate: 10°C/min; carrier gas: N2; flow 20 ml/min. The load (LC) is calculated according to the following formula:

$$\frac{\eta^3 - \text{LC}}{1 - \eta^1 - \eta^2 - \text{LC}} = \frac{\eta^{3'}}{1 - \eta^{1'} - \eta^{2'}}. \quad (2)$$

Here, η^1 is weight loss score of essential oil microcapsules in the range of 25~100°C; η^2 is weight loss score of essential oil microcapsules in the range of 100~200°C (surface residual and absorbed essential oil); η^3 is fine oil microcapsules in 200~600°C; $\eta^{1'}$ is weight loss score of the blank microcapsules in the 25~100°C segment; $\eta^{2'}$ is the weight loss score of the blank microcapsules in the 100~200°C segment; $\eta^{3'}$ is the weight loss score of the blank microcapsules in the 200~600°C segment.

2.3.3. Scanning Electron Microscopy Analysis. SEM analyzed the morphology of microcapsules on a s-3400N scanning electron microscope (Hitachi, Japan), coating microcapsule samples with a thin sputtered gold layer prior to inspection [18].

2.3.4. Comparative Studies of Stability of Essential Oil Free (EO) and Microencapsulated (MCs). Comparative studies of stability for free and microencapsulated essential oil (EO and MCs, respectively) were conducted during 24 h of monitoring time at 45°C (two replicates), taking the same quality of essential oils and microcapsules, judged by the volatile oil and residual quality of essential oils. For the preliminary studies of stability and biological activity, the microcapsule preparation selected EO: β-CD = 1 : 6.

2.4. Nematicide Bioassay

2.4.1. C. elegans Strains and Handling. The nematodes used in this study were Bristol wild-type N2. We maintained *C. elegans* and assayed them on nematode growth medium agar plates carrying a lawn of *E. coli* OP50 at 20°C. The phytochemicals were dissolved in 0.1% (v/v) DMSO. All chemicals

in the nematode growth medium plate and liquid are expressed as final concentrations.

2.4.2. Comparative Nematicide Studies. Mortality studies were conducted of the activity of (1) free FFC EO compared to (2) encapsulated EO (i.e., in MCs). The EO and MC solutions were prepared using 0.1% DMSO as a solvent. The EO-to-β-CD ratio was 1 : 6, and the loading capacity (LC) was 32.67%. Then 3.3 mg of EO and 10 mg of MCs were dissolved in 1 mL of 0.1% DMSO; in the control group, no DMSO was added. One hundred L4 *C. elegans* were placed in three separate groups, and the mortality was determined for 4 h at 30 min intervals. Three independent biological replicates were performed.

2.4.3. Oxidative Stress Resistance Assays for C. elegans. Oxidative stress assays were performed essentially as previously described. Wild-type N2 *C. elegans* was synchronized to the L4 period and pretreated with 10 mg microcapsule and 3 mg EO (LC is approximately 30%), and 0.1% DMSO as the solvent control for 48 h before exposure to $K_2Cr_2O_7$ (8 μL, 10 mM). Culturing was done for 48 h. Worms were observed every 4 h, and survival was determined by touch-provoked movement [19]. At least three independent biological replicates were performed.

2.5. Influence of Four Key Compounds of FFC EO

2.5.1. Survival Rate. The steps were similar to those described in Section 2.4.2. Four major compounds (D-limonene, γ-terpinene, *a*-pinene, and β-pinene; 5 mM each) were dissolved in 0.1% DMSO. At least three independent biological replicates were performed using at least 100 *C. elegans*.

2.5.2. Effect on Oxidative Stress Resistance. The steps were similar to those described in Section 2.4.3. Four major compounds (D-limonene, γ-terpinene, *a*-pinene, and β-pinene; 5 mM each) were used. At least three independent biological replicates were performed.

2.6. Statistical Data Analysis. For the oxidative stress, we used GraphPad Prism Software and the statistical significance of the difference between the curves was demonstrated by a log-rank test using Kaplan–Meier survival analysis. ANOVA test and subsequent Dunnett test were used to analyze the data ($P < 0.05$). Statistical analysis was performed using SPSS Statistics software (SPSS). Results are expressed as mean ± standard error of the mean (SEM). Differences were considered significant at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ levels [20].

3. Results and Discussion

3.1. Chemical Composition of EO, and Preparation and Analysis of MCs. The first step was to evaluate the chemical composition of FFC EO and then prepare and analyze MCs. The EO consists of the following: D-limonene, 47.93%; γ-terpinene, 27.14%; β-pinene, 7.70%; *a*-pinene, 4.40%. These four components account for 87.17% of the EO.

Table 1: LC and EY of microencapsulated EOs[1].

EO : β-CD	1 : 5	1 : 6	1 : 7
LC (%)	29.56 ± 1.01	32.67 ± 1.16	23.89 ± 1.62
EY (%)	96.03	96.24	95.24

[1]EO: essential oil; EY: embedding yield; LC: loading capacity.

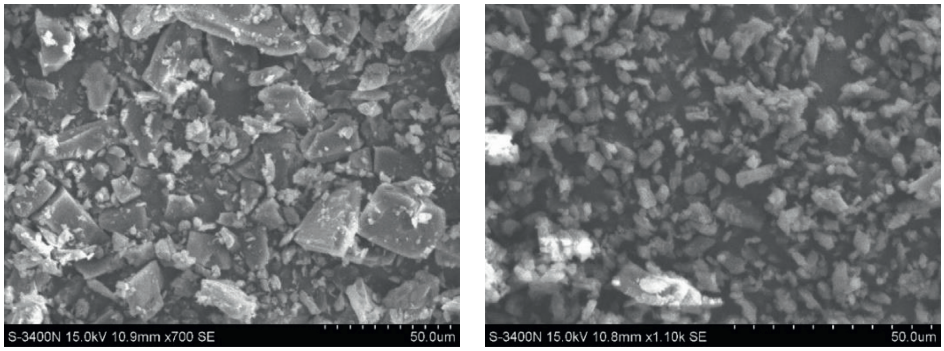
MCs prevent degradation of internal active substances and loss of activity due to external environmental factors [21]. The encapsulated EO as the ratio of β-CD increased from 1 : 5 to 1 : 7, indicating that the embedding yield (EY) at 96.24% (1 : 6) was the maximum percentage yield of the MCs (Table 1). The LC obtained by TGA also changes in accordance with the proportion and is the largest, 32.67%, at 1 : 6 (Table 1). This result may be due to the higher ratio of leaching from the internal aqueous phase volume of the primary water/oil emulsion to the external aqueous phase volume of the secondary water/oil/water emulsion [22].

3.2. Capsule Morphology. To characterize the morphology and size distribution of the capsules, a scanning electron microscope (SEM) of the samples was performed [23]. The SEM micrographs of β-CD and microcapsules with EO were depicted in Figure 1. β-CD was irregular in shape, dispersed after dissolution, and was a crystal structure. There were observed differences in the shape of β-CD and microcapsules and the embedded microcapsules were granular. The diameter of the particles in all the prepared microcapsules ranged from 10 um to 50 um.

3.3. Microcapsule Stability. We conducted comparative studies of the stability for free and microencapsulated EO over the course of 24 h at 45°C [2]. We calculated the percentage retention of EO in microcapsules as follows: $EO_{\text{at elapsed time}} \times 100/EO_{\text{at zero time}}$. Under these experimental conditions, we detected a very rapid liberation of free EO: less than 15% retention over 24 h (Figure 2). In contrast, MCs exhibited very slow liberation. Thus, in these experimental conditions, MCs exhibited sustained release of EO and were stable.

3.4. Biological Nematocide Assays

3.4.1. Mortality Rate of EO and MCs. Plant EOs and extracts directly hinder nematode survival [24]. The free-EO-induced (3.3 mg/mL) mortality rate was as follows: 2 h, 89%; 4 h, 99%. We also conducted two independent experiments using free EO (3.3 mg/mL), on different days, to study the bioassay's reproducibility and select the appropriate bioassay duration. We observed a very rapid response, a plateau (maximum response) before 2 h of incubation, in both independent experiments for free EO. In contrast, we observed a slower response for MCs (10 mg/mL). We observed considerable mortality differences for free EO and MC treatments at 2 h (97% and 37%, respectively) and 4 h (100%



(a) (b)

Figure 1: Scanning electron microscope micrographs obtained. (a) Beta-cyclodextrin (β-CD). (b) Microcapsules with essential oil (EO).

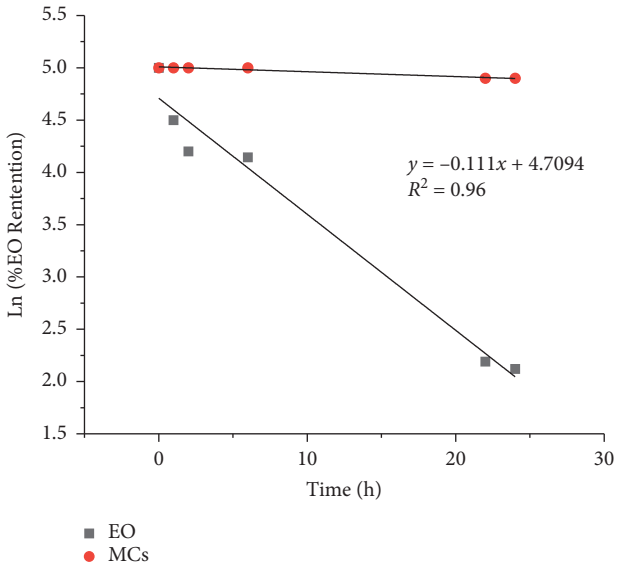


Figure 2: Comparative study of stability for total essential free oil (EO) and microencapsulated essential oil (MCs).

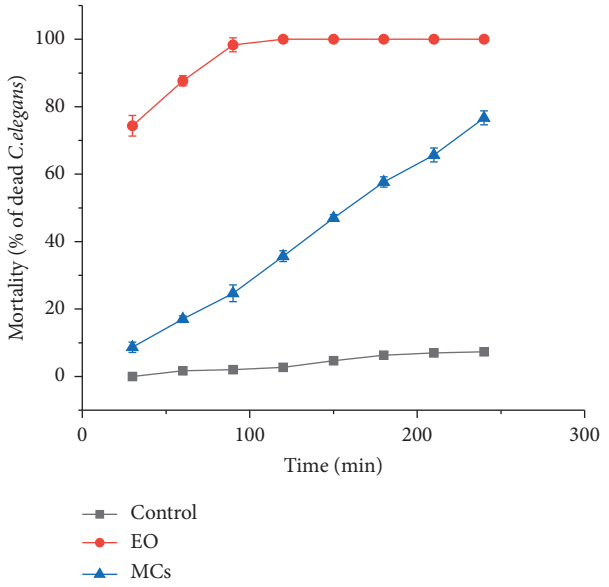


Figure 3: Average mortality (%) in comparative nematicide activity of essential oil (EO) and microcapsules (MCs). Results show at least three independent biological replicates. Error bars represent the standard error and differences compared to the control.

and 77%, respectively). Nematode mortality reached 100% within 4.5 h. Control untreated (0.1% DMSO) experiments showed 7.3% dead nematodes at 4 h (Figure 3).

3.4.2. Oxidative Stress Resistance. We used $K_2Cr_2O_7$ as an oxidative stress inducer because it is a heavy metal oxidizer. It can undergo intracellular redox cycling with superoxide radical (O2•) production in *C. elegans* and is commonly used to generate intracellular oxidative stress in *C. elegans* [25].

Pretreatment with free EO and pretreatment with MCs both induced considerable oxidative stress (Figure 4). EO contains a variety of compounds that might exert various biological activities that reduce *C. elegans*' antioxidant activity. The MCs behaved in a similar manner as free EO.

3.5. Effects of Four Compounds

3.5.1. Survival Rate. Monoterpenes such as sabinene—a volatile compound of plants—help plants defend against herbivores and plant pathogens, thus showing larvicide and antimicrobial activity [26]. The nematode 24 h survival rate was as follows: D-limonene, 30.33%; γ-terpinene, 34%; a-pinene, 12%; β-pinene, 10% (Figure 5). Therefore, the compounds that comprise FFC EO had obvious nematicidal effects. The MCs loaded with individual EOs killed nematodes somewhat slower than the corresponding free EO compounds, possibly due to slow sustained release.

3.5.2. Oxidative Stress Resistance. Different components like monoterpenoids are responsible for insecticidal properties and physiological discrepancies of insects [27, 28]. As shown in Figure 6, D-limonene, γ-terpinene, a-pinene, and β-pinene all affect the oxidative stress response of *C. elegans*, which shortens lifespan.

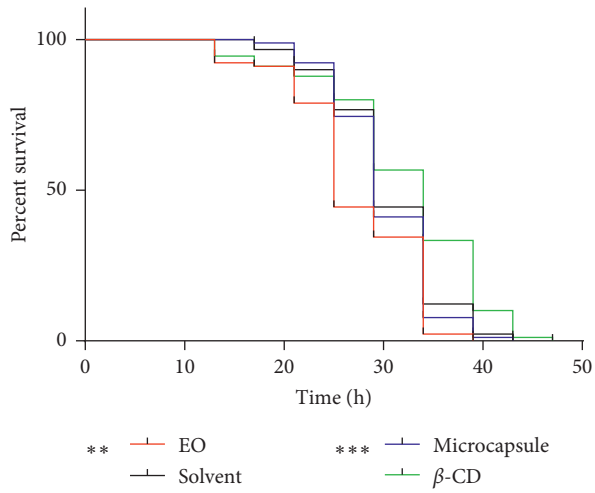


Figure 4: Essential oil of flesh fingered citrons and microcapsules affect the oxidative stress resistance of wild-type *C. elegans*. Results include at least three independent biological replicates. Statistical significance of the difference between the curves was demonstrated by a log-rank test using Kaplan–Meier survival analysis. Error bars represent the standard error, and differences compared to the solvent (0 mg/ml, 0.1% DMSO) were considered significant at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ by one-way ANOVA and the LSD post hoc test.

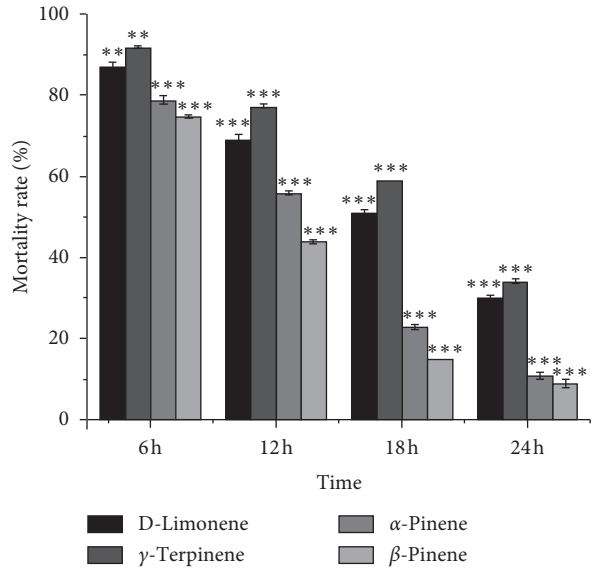


Figure 5: Four compounds in the flesh fingered citrons essential oil affect the survival rate on *C. elegans*. Results include at least three independent biological replicates. Error bars represent the standard error, and differences compared to the control (for each column of 0 mg/ml, 0.1% DMSO) were considered significant at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ by one-way ANOVA and the LSD post hoc test.

4. Conclusions

In recent years, researchers have used plant EOs and extracts to hinder nematode survival [29]. Given that EOs are potential pest control agents, environmental impact assessments of EOs have received much attention [30]. Most plant extracts used in pest control inhibit—rather than kill—insects and nematodes, e.g., inhibit feeding. We used MCs to encapsulate EO extracted from FFC as a plant-derived nematicide to inhibit *C. elegans*. We found that FFC EO is a complex mixture of terpenes (monoterpenes, sesquiterpene, and derivatives) that degrade rapidly, are selective, exhibit low mammalian toxicity, and have a minimal environmental impact [31]. We used β-CD to embed the EO of FFC and found that an LC of 1 : 6 is optimal. MCs exhibit good sustained release.

After 4 h, the nematode mortality rate induced by EO and MCs was 100% and 77%, respectively. Due to the strong volatility of EOs, the residual activity is short, and repeated application is required. However, MCs exhibit controlled release and stabilise EOs prior to release. Furthermore, oxidative stress experiments showed that the EO and MCs shorten nematode survival times by reducing antioxidant activity. Four isolated individual terpene compounds considerably increased mortality, indicating that they are the source of nematicidal activity. The MCs also play a nematicidal role by slowly releasing monoterpenes. In conclusion, our microencapsulation procedure will be useful for preparing plant-derived pesticides and controlled release of active ingredients. In this manner, EO is protected from the external environment during product application and is slowly released, providing a more persistent nematicidal effect compared to no encapsulation [2].

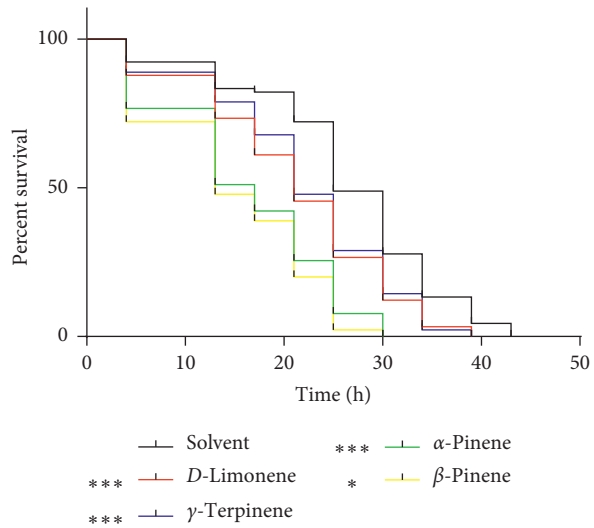


Figure 6: Four components in the flesh fingered citrons essential oil affect the oxidative stress resistance of wild-type *C. elegans*. At least three independent biological replications were performed, and the statistical significance of the differences between curves was demonstrated by a log-rank test using Kaplan–Meier survival analysis. Error bars represent the standard error, and differences compared to the solvent (0 mg/ml, 0.1% DMSO) were considered significant at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ by one-way ANOVA and the LSD post hoc test.

Data Availability

The numerical data used to support the findings of this study are included within the article.

Additional Points

Highlights. (1) A plant-derived nematicide is proposed. (2) The embedding conditions of flesh fingered citron (FFC) essential oil (EO) microcapsules (MCs) and the MC's slow-release effect were explored. (3) The authors investigated the nematicidal mechanism of the four main components of FFC EO. (4) Our microencapsulated EO can replace synthetic nematicides, in that MCs can store EO for long periods of time and are environmentally friendly.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] P. Majumder, H. A. Mondal, and S. Das, "Insecticidal activity ofArum maculatumTuber lectin and its binding to the glycosylated insect gut receptors," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 17, pp. 6725–6729, 2005.

[2] A. López, S. Castro, M. J. Andina et al., "Insecticidal activity of microencapsulated Schinus molle essential oil," *Industrial Crops and Products*, vol. 53, pp. 209–216, 2014.

[3] J. Clardy and C. Walsh, "Lessons from natural molecules," *Nature*, vol. 432, no. 7019, pp. 829–837, 2004.

[4] J. S. Raut and S. M. Karuppayil, "A status review on the medicinal properties of essential oils," *Industrial Crops and Products*, vol. 62, pp. 250–264, 2014.

[5] A. K. Tripathi, S. Upadhyay, M. Bhuiyan, and P. A. Bhattacharya, "Review on prospects of essential oils as biopesticide in insect-pest management," *Journal of Pharmacognosy and Phytotherapy*, vol. 1, pp. 52–63, 2009.

[6] M. B. Isman, "Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world," *Annual Review of Entomology*, vol. 51, no. 1, pp. 45–66, 2006.

[7] C. Regnault-Roger, "The potential of botanical essential oils for insect pest control," *Integrated Pest Management Reviews*, vol. 2, no. 1, pp. 25–34, 1997.

[8] C. Regnault-Roger, C. Vincent, and J. T. Arnason, "Essential oils in insect control: low-risk products in a high-stakes world," *Annual Review of Entomology*, vol. 57, no. 1, pp. 405–424, 2012.

[9] D. Chouchi, D. Barth, E. Reverchon, and G. Della Porta, "Supercritical CO2 desorption of bergamot peel oil," *Industrial & Engineering Chemistry Research*, vol. 34, no. 12, pp. 4508–4513, 1995.

[10] E. Shaaya, M. Kostjukovski, J. Eilberg, and C. Sukprakarn, "Plant oils as fumigants and contact insecticides for the control of stored-product insects," *Journal of Stored Products Research*, vol. 33, no. 1, pp. 7–15, 1997.

[11] E. N. Jesser, J. O. Werdin-González, A. P. Murray, and A. A. Ferrero, "Efficacy of essential oils to control the Indian meal moth, Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae)," *Journal of Asia-Pacific Entomology*, vol. 20, no. 4, pp. 1122–1129, 2017.

[12] R. A. Cloyd and H. Chiasson, "Activity of an essential oil derived from Chenopodium ambrosioides on greenhouse insect pests," *Journal of Economic Entomology*, vol. 100, no. 2, pp. 459–466, 2007.

[13] R. V. Tonon, C. R. F. Grosso, and M. D. Hubinger, "Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying," *Food Research International*, vol. 44, no. 1, pp. 282–289, 2011.

[14] M. M. M. Specos, J. J. García, J. Tornesello et al., "Microencapsulated citronella oil for mosquito repellent finishing of cotton textiles," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 104, no. 10, pp. 653–658, 2010.

[15] A. Bouyanfif, S. Liyanage, E. Hequet, N. Moustaid-Moussa, and N. Abidi, "Review of FTIR microspectroscopy applications to investigate biochemical changes in *C. elegans*," *Vibrational Spectroscopy*, vol. 96, pp. 74–82, 2018.

[16] Y. Li, C. Wu, T. Wu et al., "Preparation and characterization of citrus essential oils loaded in chitosan microcapsules by using different emulsifiers," *Journal of Food Engineering*, vol. 217, pp. 108–114, 2018.

[17] S. Banerjee, L. Siddiqui, S. S. Bhattacharya et al., "Interpenetrating polymer network (IPN) hydrogel microspheres for oral controlled release application," *International Journal of Biological Macromolecules*, vol. 50, no. 1, pp. 198–206, 2012.

[18] D. Zhou, Y. Pan, J. Ye, J. Jia, J. Ma, and F. Ge, "Preparation of walnut oil microcapsules employing soybean protein isolate and maltodextrin with enhanced oxidation stability of walnut oil," *LWT-Food Science and Technology*, vol. 83, pp. 292–297, 2017.

[19] G. J. Lithgow and G. A. Walker, "Stress resistance as a determinate of *C. elegans* lifespan," *Mechanisms of Ageing and Development*, vol. 123, no. 7, pp. 765–771, 2002.

[20] A. Pant, S. K. Saikia, V. Shukla, J. Asthana, B. A. Akhoon, and R. Pandey, "Beta-caryophyllene modulates expression of stress response genes and mediates longevity in *Caenorhabditis elegans*," *Experimental Gerontology*, vol. 57, pp. 81–95, 2014.

[21] N. Juran, K. Jin, S. K. Jin, S. C. Young, T. C. Suk, and P. Juhyun, "Microencapsulation by pectin for multi-components carriers bearing both hydrophobic and hydrophilic active agents," *Carbohydrate Polymers*, vol. 182, pp. 172–179, 2018.

[22] B. Subham, C. Pronobesh, G. Animesh, G. Danswrang, K. Sanjeev, and V. Vijay, "Influence of process variables on essential oil microcapsule properties by carbohydrate polymer–protein blends," *Carbohydrate Polymers*, vol. 93, pp. 691–697, 2013.

[23] M. Ahmad, S. Qureshi, S. Maqsood, A. Gani, and F. A. Masoodi, "Micro-encapsulation of folic acid using horse chestnut starch and β-cyclodextrin: microcapsule characterization, release behavior & antioxidant potential during GI tract conditions," *Food Hydrocolloids*, vol. 66, pp. 154–160, 2017.

[24] S. Morteza, Z. Arash, S. Najmeh, and S. Leila, ""Effects of α-pinene, trans-anethole, and thymol as the essential oil constituents on antioxidant system and acetylcholine esterase of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae)," *Pestic*," *Biochemistry and Physiology*, vol. 150, pp. 40–47, 2018.

[25] C.-W. Yu, W.-H. Li, F.-L. Hsu, P.-L. Yen, S.-T. Chang, and V. H.-C. Liao, "Essential oil alloaromadendrene from mixed-type Cinnamomum osmophloeum leaves prolongs the lifespan in *Caenorhabditis elegans*," *Journal of Agricultural and Food Chemistry*, vol. 62, no. 26, pp. 6159–6165, 2014.

[26] S. S. Cheng, C. Y. Lin, M. J. Chung, Y. H. Liu, C. G. Huang, and S. T. Chang, "Larvicidal activities of wood and leaf essential oils and ethanolic extracts from Cunninghamia konishii Hayata against the dengue mosquitoes," *Industrial Crops and Products*, vol. 47, pp. 310–315, 2013.

[27] A. Dinesh-Kumar, E. Srimaan, M. Chellappandian et al., "Target and non-target response of Swietenia Mahagoni Jacq.

chemical constituents against tobacco cutworm Spodoptera litura Fab. and earthworm, Eudrilus eugeniae Kinb," *Chemosphere*, vol. 199, pp. 35–43, 2018.

[28] N. Kumrungsee, W. Pluempanupat, O. Koul, and V. Bullangpoti, "Toxicity of essential oil compounds against diamondback moth, Plutella xylostella, and their impact on detoxification enzyme activities," *Journal of Pest Science*, vol. 87, no. 4, pp. 721–729, 2014.

[29] M. Shahriari, A. Zibaee, N. Sahebzadeh, and L. Shamakhi, "Effects of α-pinene, trans-anethole, and thymol as the essential oil constituents on antioxidant system and acetylcholine esterase of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae)," *Pesticide Biochemistry and Physiology*, vol. 150, pp. 40–47, 2018.

[30] N. Z. Dimetry, "Different plant families as bioresource for pesticides," in *Advances in Plant Biopesticides*, D. Singh, Ed., Springer, Berlin, Germany, pp. 1–20, 2014.

[31] R. A. Cloyd, S. R. Keith, and C. L. Galle, "Effect of the insect growth regulator novaluron (Pedestal) on silverleaf whitefly reproduction," *HortTechnology*, vol. 14, no. 4, pp. 551–554, 2004.