DOI:10.1021/jf9023118

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Structural Characterization of Nanoparticles Loaded with Garlic Essential Oil and Their Insecticidal Activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

Feng-Lian Yang,[†] Xue-Gang Li,[‡] Fen Zhu,[†] and Chao-Liang Lei*,[†]

[†]Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory, Huazhong Agricultural University, Wuhan 430070, People's Republic of China, and [‡]Department of Chemistry, College of Science, Huazhong Agricultural University, Wuhan 430070, People's Republic of China

The aim of this work was to characterize polyethylene glycol (PEG) coated nanoparticles loaded with garlic essential oil and to evaluate their insecticidal activity against adult *Tribolium castaneum*. Preparation of nanoparticles was carried out using the melt-dispersion method, a very simple, convenient, and low-cost technique. The oil-loading efficiency could reach 80% at the optimal ratio of essential oil to PEG (10%). The morphology results and nanoparticle size showed that the nanoparticles had a round appearance and good dispersion, <240 nm in the average diameter, characterized by transmission electron microscope and dynamic light scattering, respectively. The abundance and percentage content of the major components did not show any significant difference between free and nanoencapsulated oil when analyzed by gas chromatography−mass spectrometry. The control efficacy against adult *T. castaneum* remained over 80% after five months, presumably due to the slow and persistent release of the active components from the nanoparticles. In contrast, the control efficacy of free garlic essential oil at the similar concentration (640 mg/kg) was only 11%. This indicates that it is feasible to use the PEG coating nanoparticles loaded with garlic essential oil to control the store-product pests.

KEYWORDS: Nanoparticles; garlic essential oil; polyethylene glycol (PEG); loading efficiency; structural characterization; insecticidal activity

INTRODUCTION

Garlic (*Allium sativum* L.) is not only a food ingredient widely used in gastronomy, it has also been used for over 4000 years as a medicinal plant for a variety of ailments including headaches, bites, intestinal worms, and tumors (*1*). To this day, the medicinal use of garlic remains popular all over the world (*2*−*4*), and its strong insecticidal activity has also been demonstrated by several studies (*5*−*9*). Several garlic products such as garlic essential oil and garlic powder are available in the international market for use mainly as pest control. However, the volatility, strong odor, and insolubility in water of these extracts remain the main disadvantages of garlic essential oil (*1*), thus, directly affecting the duration of its insecticidal effectiveness and the taste of treated food when it is used as a grain protectant.

Alternative formulations such as microencapsulation are therefore being developed to increase the persistence of bioactive plant essential oils by reducing their volatilization and slowing down their rate of degradation in the environment (*10*). It is reported that garlic extract-loaded microcapsules can protect bioactive compounds from volatilizing, prolong shelf life, and decrease certain characteristic odors and aftertaste (*1*, *11*). However, no specific research to evaluate the effect of encapsulating garlic essential oil has been performed to demonstrate these advantages.

Microencapsulation is a technique in which a membrane encloses small particles of solid, liquid, or gas with the objective of offering protection to the core material from adverse environmental conditions, such as undesirable effects of light, moisture, and oxygen while avoiding drawbacks such as odor and volatility (*12*). Controlled release through microencapsulation has been shown to increase the insecticidal efficacy of some pesticides used on field crops (*13*) and in warehouses against stored-product pests (*14*). Pesticide microcapsules represent one of the important development trends for future pesticide formulations.

The rapid development of nanotechnology has resulted in its application in fields such as computer electronics, communication and energy production, paving the way for further application in food technology (*15*). Presently, nanotechnology has likewise received considerable attention in the formulation of therapeutic drugs in medicine. Because polymeric nanoparticles manifest great potential in drug delivery systems as a result of their controlled- and sustained-release properties and their submicrometer size, with diameters ranging from 1 to 1000 nm (*16*). These features further offer them with a number of distinct advantages over microparticles (*17*). There have been several reports of nanotechnology being used in the food and agriculture field, but its applications to the agriculture and food sector remain

*To whom correspondence should be addressed. Tel./Fax +86-27-87287207. E-mail: ioir@mail.hzau.edu.cn.

 Published on Web 10/16/2009

relatively recent and, thus, limited compared to their use in drug delivery and pharmaceuticals (*15*). There is also little information about pesticide nanocapsules applied in warehouses as a means to protect stored grain from infection caused by stored-product pests.

Hence, based on the advantages of nanoparticles over microparticles, the aim of this work was to characterize nanoparticles containing garlic essential oil prepared with biocompatible polyethylene glycol (PEG) using the melt-dispersion method. This work also analyzed the composition of garlic essential oil pre- and postencapsulation by gas chromatography−mass spectrometry (GC-MS) and evaluated the insecticidal activity of nanoparticles against adult *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae).

MATERIALS AND METHODS

Compounds and Commodities. Garlic essential oil (purity >85%, containing three major constituents, about 35% of diallyl disulfide, 42% of diallyl trisulfide, and 16% of diallyl sulfide), stored at 4 °C, was purchased from Xunyang Ltd. Co., Guangdong, China; polyethylene glypresentcol 6000 (PEG) was supplied by Shanghai Zhuokang Chemical Ltd., Co., China.

Fresh rough rice grown without insecticides was harvested from experimental fields in Huazhong Agricultural University, Wuhan, China. All grain was sterilized in an oven at 60 °C for 3 h before experimentation. The initial moisture of the grain used was approximately 13%.

Insects. Red flour beetles, *T. castaneum*, used in this study were obtained from laboratory cultures, reared on milled rice mixed with yeast (10:1, w/w), maintained in the dark in incubators at 30 ± 1 °C and 70−80% r.h. Mixed-sex adults used in tests were 2−3 weeks posteclosion and starved for 24 h before experiment.

Preparation for Nanoparticles Loaded with Garlic Essential Oil. Nanoparticles loaded with garlic essential oil were prepared using the melt-dispersion method according to the method of Peng et al. (*18*). Several parts of PEG6000 (100 g per part) were heated separately at 65 °C. After being melted, 2.5, 5.0, 7.5, and 10.0 mL of garlic essential oil were separately mixed with melted PEG and stirred lightly with a glass rod to ensure even distribution of the mixture. The mixture was ground completely in a mortar box after being cooled naturally at 25 °C and then sieved using a sieve mesh 200. The powders were placed in airtight, self-sealable polyethylene pouches and stored at 25 °C in desiccators containing calcium chloride to prevent moisture absorption prior to further experiments.

Garlic Essential Oil-Loading Efficiency. The standard curve of concentration versus absorbency for garlic essential oil was drawn according to the method of Peng et al. (*18*) but with slight modifications. Briefly, aliquots of garlic essential oil were diluted in absolute ethanol by serial dilution method to obtain a series of concentrations. The colorimetric assay at 325 nm was carried out for absorbency of the respective concentration using a UV−visible spectrophotometer (U-3010, HITACHI), which was used to draw the standard curve.

Nanoparticle samples (0.1 g per part) containing different quantities of garlic essential oil were dissolved separately in 2 mL of absolute ethanol. The mixture of nanoparticles and ethanol was heated in a closed centrifugal tube for 5−6 h in a hot water heater (40 °C) until completely dissolved. The absorbency of the solution was then determined at 325 nm by UV−visible spectrophotometer, and the result was compared to that of the standard curve. The loading efficiency (LE) of oil-loaded nanoparticles was calculated using the following equation: $\%LE = [A \times B/C] \times 100$, where A is the content of garlic essential oil loaded with 0.1 g nanoparticles, B is the total quantity of PEG (that is 100 g in the test), and C is the original quantity of garlic essential oil added into the total quantity of PEG, which is 2.5, 5.0, 7.5, and 10.0 mL, respectively. Each test was repeated three times.

Characterization of Nanoparticles. Nanoparticles prepared with the 10% optimal ratio of garlic essential oil to PEG were selected and characterized. Some nanoparticles were quantified and dispersed in absolute ether for 10 min in sonication dispersion, forming a homogenized solution. A drop of the homogenized solution was transferred onto a carbon-coated copper grid, followed by negative staining with a phosphotungstic acid solution (2%, pH = 6.7) for 1 min. After the replica was dried at room temperature (25 °C), the image was visualized with a transmission electron microscope (TEM) at 80 KV and with a Gatan 832 CCD camera (Hitachi H-7650, Japan). The average size and size distribution were determined using a dynamic light scattering particle size analyzer (LB-550, Horiba, Japan) at 25 °C, the measuring range was from 1 nm to 6 μm, and the light source was a 650 nm laser diode of 5 mV. The 0.2 g samples of about 20.0 mL absolute ether dispersions were measured directly without any pretreatment. The morphology and size of the nanoparticles were obtained from the measurements of three batches of nanoparticles.

Analysis of Composition of Garlic Essential Oil at Pre-/Post-Encapsulation. The samples prepared with a 10% optimal ratio of oil to PEG was sampled at 0 and 5 months after encapsulation to analyze the compositions of nanoencapsulates. Extraction of the encapsulated material was made using absolute ether and a limited quantity of distilled water. Quantification of the extracted garlic essential oil and free garlic essential oil was carried out by GC-MS (Agilent 7890A/5975C, U.S.A.). The GC column was a 30 m×0.25 mm (i.d.) fused silica capillary column coated with 5% phenyl-methylpolysiloxane using a HP-5MS (df = 0.25 μm; Agilent J&W Scientific, U.S.A.). The GC conditions were as follows: initial oven temperature was held at 60 °C for 1 min, and then programmed to 240 at 5 °C/min. It was maintained at this temperature for 10 min. The injector temperature and the ion source temperature were 250 and 200 °C, respectively. Helium was used as the carrier gas at a rate of 1 mL/min. The samples (1 μL) were injected neat with a split ratio of 1:50. The effluent of the GC column was introduced directly into the source of the MS. Spectra were obtained in the EI mode using an ionization energy of 70 eV. The sector mass analyzer was set to scan from 29 to 400 amu for 3 s. Compounds were identified by comparing the mass spectrum of each peak with those of authentic samples in a mass spectra library (The Wiley Registry of Mass Spectra Data, 6th ed.) and were confirmed by comparing the retention times obtained by GC with those of authentic samples. Each extraction from three parts of samples with the same content of essential oil was measure one time separately.

Insecticidal Bioassay of Nanoparticles against Adult *T. castaneum*. Many samples of 100 g rough rice each containing 2% milled rice (11% m.c.) in 250 mL jars were treated with doses of 160, 320, 480, and 640 mg/kg of free garlic essential oil and 2000, 4000, 6000, and 8000 mg/kg of nanoparticles (equal to 160, 320, 480, and 640 mg/kg of free garlic essential oil), respectively. Afterward, all jars were sealed and then shaken to spread the compounds throughout the rice. There was a series of untreated lots served as controls for the treatments of free garlic oil and nanoparticles loaded with garlic oil.

Jars each containing 100 g treated grain were stored in chambers at 28 ± 1 °C, 75 ± 5% r.h. Residual bioassays were conducted after the grains were stored for 1 day (0 month), as well as for 1, 2, 3, 4, and 5 months. Bioassays were as follows: 60 insects were introduced into each jar containing 100 g grains treated with different concentrations of garlic essential oil or nanoparticles and exposed for 5 d. After the 5 d treatment, the grain was sifted for weevils, which were classified as active, knocked down, or immobile, according to the method of Arthur (*14*), and then counted. Active insects were regarded as "surviving", while knocked down and immobile insects were classified as "not surviving" after exposure to the treated grain.

The treatments were repeated three times by preparing new treated and untreated lots each time. For each treatment, the number of jars (containing 100 g of grain per jar) totaled 30 at the whole stored time for one of both formulations of garlic oil, including the control jars.

Statistical Analyses. The mortality data of adult *T. castaneum* were corrected for mortality in controls and transformed to arcsine square root values for analysis of variance (ANOVA). One-/two-way ANOVA and Duncan's multiple-range test (DMRT) were used to determine the means, and for their comparison, $P = 0.05$, using the procedure of SPSS V13.0. The linear regression model for the corrected mortality of test pests treated with different doses was documented using Microsoft Excel 2003.

RESULTS AND DISCUSSION

PEG has a simple, helical molecular structure that possesses a number of outstanding physicochemical and biological properties,

including hydrophilicity, solubility in water and organic solvents, and lack of toxicity (*19*). Moreover, some research has reported that a PEG coating around the nanocarrier helps to improve stability in biological fluids and, as a consequence, facilitates the transport of bioactive macromolecules across the intestinal and nasal epithelia (*20*−*22*).

Taking into consideration the properties of PEG, this research was thus carried out to determine the most suitable characteristics of the PEG coating nanoparticles with respect to their use as garlic essential oil carriers and to control stored-product pests effectively. The primary objective for the research findings was to determine the most suitable formulations for phytochemicals when effectively applied in the agriculture and food sector.

Preparation of Nanoparticles Loaded with Garlic Essential Oil and Evaluation of Oil-Loading Efficiency. Usually, there are many methods to produce a nanocapsule/particle, such as the spray-drying method (*23*), complex coacervation (*24*), interfacial polymerization (*25*), emulsion-diffusion method (*26*), and so on. Although these methods mentioned above are widely used to prepare for nanoparticles/capsulates in many disciplines, the production procedure for each method is too complex and needs too much time. Moreover, some of them must reach a higher temperature in the production process, for instance, the temperature should be above 100 °C during the process of preparation for nanoparticles/capsulates using the spray-drying method. In contrast, the melt-dispersion method was used to prepare the PEG coating nanoparticles, a procedure that is very simple and can be easily compared to other nanotechnologies. Moreover, the temperature was set much lower during the melt-dispersion encapsulation process in order to protect the constituents of garlic essential oil better from volatilizing, especially for the components with a low boiling point. Additionally, the time for preparing the PEG coating nanoparticles loaded with garlic essential oil was very short. The simple procedure and short preparation time facilitated the production process of nanoparticles containing garlic essential oil.

Table 1. Quantity and Loading Efficiency of Garlic Essential Oil in PEG Coating around Nanoparticles

ratio of garlic essential oil to PEG	quantity of encapsulated garlic essential oil per 100 g nanoparticles (mL) ± SE	% loading efficiency ± SE
2.5 mL/100 g	0.99 ± 0.00	39.79 ± 5.25
5 mL /100 g	2.98 ± 0.01	59.54 ± 3.23
7.5 mL /100 g	5.20 ± 0.02	69.39 ± 3.07
10 mL /100 g	8.01 ± 0.05	80.46 ± 4.94

Oil encapsulation efficiency is a critical factor for nanoparticles. A good nanocarrier should have high oil encapsulation efficiency. The amount of garlic essential oil encapsulated in particles was obtained from the standard curve of the concentration of garlic essential oil versus its absorbency ($y = 1.1305x + 0.7102$, $R^2 = 0.9689$) at 325 nm. **Table 1** shows the oil-loading efficiency in the PEG coating nanoparticles. The oil-loading efficiency was positively correlated to the amount of garlic essential oil, that is, it increased with the increasing ratio of garlic oil to PEG. Oil-loading efficiency reached 80% at the 10% optimal ratio of garlic oil to PEG. The results suggest that the melt-dispersion method of preparing nanoparticles of garlic essential oil coated with PEG is feasible, with an 80% high loading efficiency at a 10% ratio of oil to PEG.

Table 2. Content Analysis of Major Constituents of Garlic Essential Oil Pre-/Post-Encapsulated by Mass Spectral (%)[a]

	diallyl disulfide	diallyl trisulfide	diallyl sulfide
free garlic essential oil	34.68 ± 0.42a	41.75 ± 0.99a	15.75 ± 0.53a
garlic essential oil post-encapsulated, month 0	32.31 ± 0.65a	39.83 ± 1.24a,b	18.43 ± 0.73b
garlic essential oil post-encapsulated, month 5	26.67 ± 0.34b	37.40 ± 0.59b	25.66 ± 0.76c
F	68.026	4.957	56.284
n	2.8	2.8	2.8
sig.	<0.0001	0.054	<0.0001

[a] Means within a column followed by different letters are significantly different in ANOVA ($P < 0.05$, DMRT).

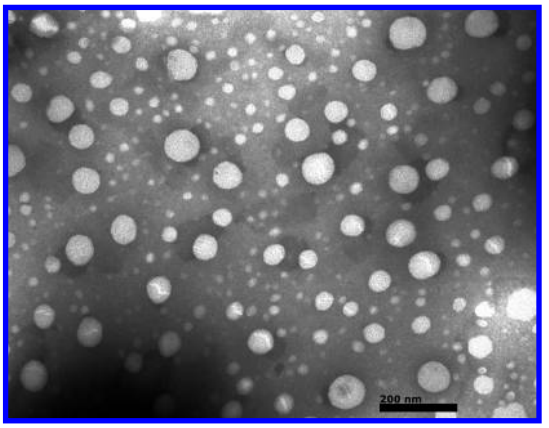

Figure 1. TEM image of PEG coating nanoparticles loaded with garlic essential oil.

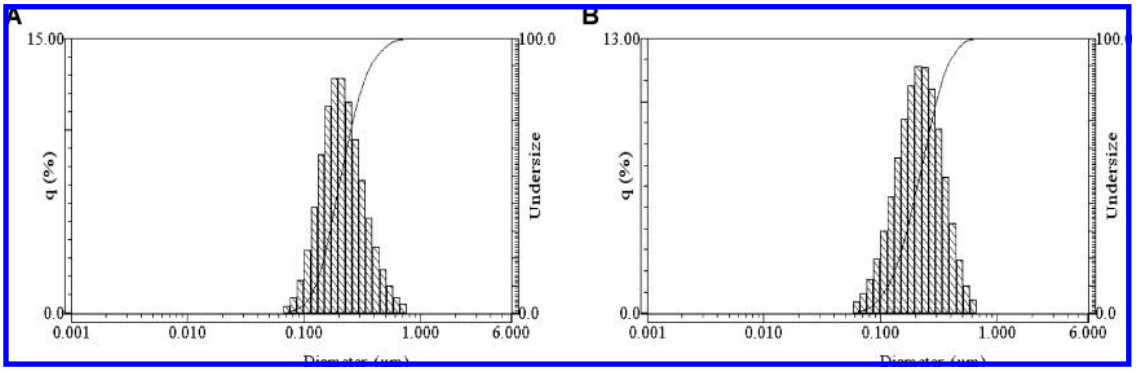

Figure 2. Size distributions of PEG coating nanoparticles loaded with garlic essential oil measured with dynamic light scattering: (A) stored 0 month; (B) stored 5 months; "q" refers to "number frequency of particle size" and "Undersize" means "cumulative distribution of particle size <6 μm" in figures; "left *y* axis" represents "bar" and "right *y* axis" represents "line".

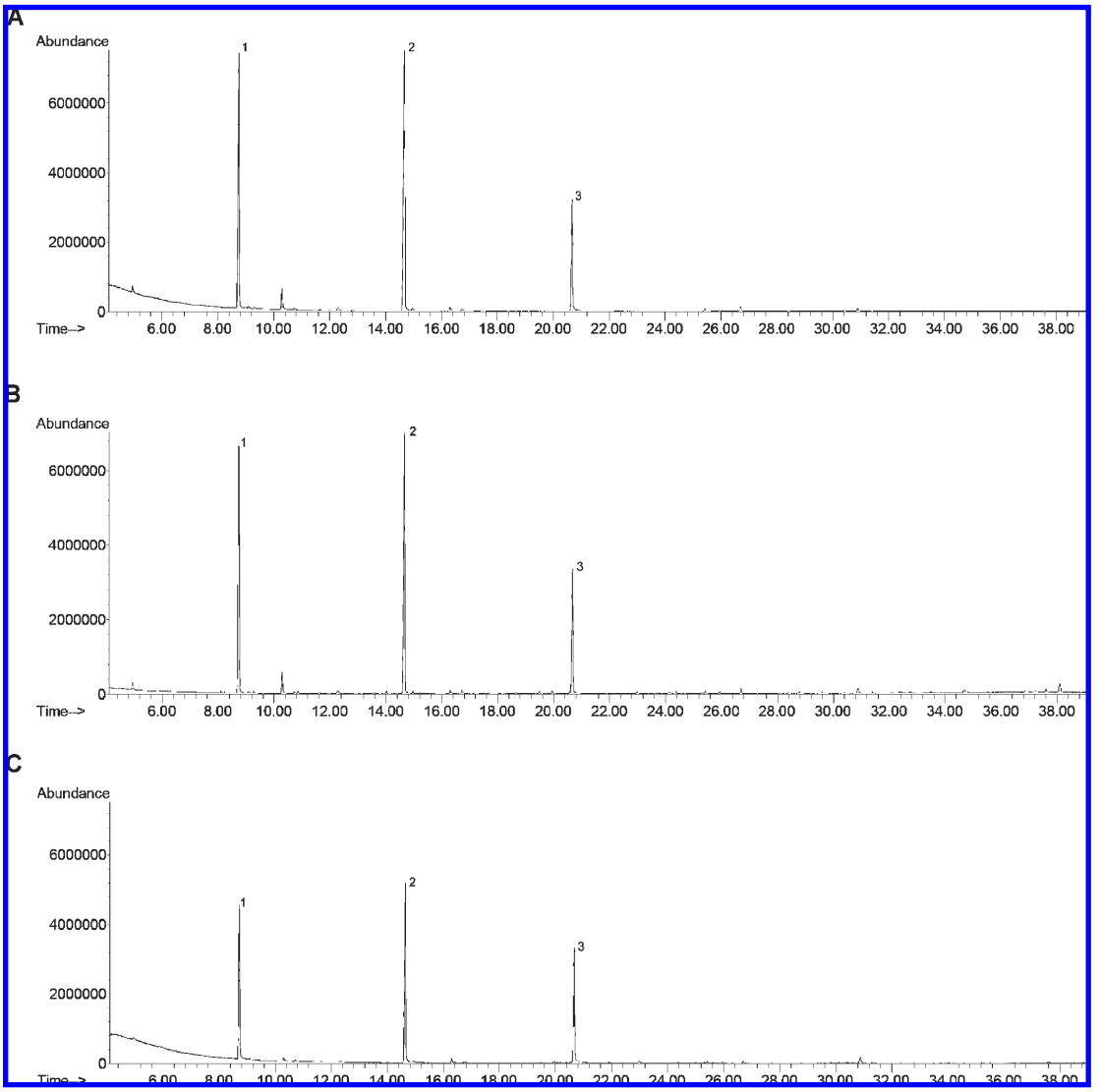

Figure 3. Chromatograms of garlic essential oil and nanoencapsulated garlic essential oil submitted to a stability study at 25 °C: (A) garlic essential oil pre-encapsulated; (B) garlic essential oil post-encapsulated, month 0; (C) garlic essential oil post-encapsulated, month 5. Peaks: 1, diallyl disulfide; 2, diallyl trisulfide; 3, diallyl sulfide.

Characterization of Nanoparticles. The morphology of nanoparticles, at the 10% ratio of garlic essential oil to PEG, was visualized using TEM. **Figure 1** shows the particles appearing round and in good dispersion, with the external surfaces presenting no apparent cracks or porosity, aspects that are indicative of good protection of the core material. The distribution size and the mean size of the nanoparticles stored for month 0 and month 5 are shown in **Figure 2**. Nanoparticle solutions revealed a unimodal size distribution. Comparing the results of mean sizes of nanoparticles at the two storage times it could be seen that the nanoparticles were very stable. There was no difference between the mean size and the size distribution of the nanoparticles, one of which was 233 ± 108 nm at month 0 and the other at 235 ± 105 nm at month 5.

Comparing the results of nanoparticles obtained by TEM and dynamic light scattering, the size of the former is revealed to be smaller than that of the latter (**Figures 1** and **2**, respectively). The reason could be caused by the sampling process prepared for TEM. During the process of visualizing an image on the nanoparticles, the heat generated by electron beams could increase the melting point of PEG (60 °C), which may have softened and melted the PEG wall.

It is reported that the ultimate determination of nanocapsule size depends on three factors: polymer concentration in the organic phase, solvent polarity, and internal/external phase ratio (*27*). In this study, the production of stable nanoparticles with nano sizes was attributed to the structural properties of PEG and the 10% optimal ratio of essential oil to PEG.

Composition Analysis of Garlic Essential Oil at Pre-/Post-Encapsulation. In addition to the volatility and diffusion of the core material through the wall of the PEG-coated nanocapsule, the action of other factors from the environment (e.g., temperature and oxygen) can catalyze reactions, leading to the decrease in the content of the compound originally encapsulated (*28*). On the same note, this decrease could be attributed to the susceptibility of the core material to the oxidized degradation process (*12*).

Analysis of the results from GC-MS indicates there were no significant chemical variations between pre-encapsulated and post-encapsulated garlic essential oil stored at month 0 and month 5, including three major constituents, specifically, diallyl disulfide, diallyl trisulfide, and diallyl sulfide (**Table 2**). Meanwhile, there were no other oxidized derivatives found, except in the variations of the content of the three major constituents (**Figure 3**, **Table 2**). The results further indicate that nanoparticles

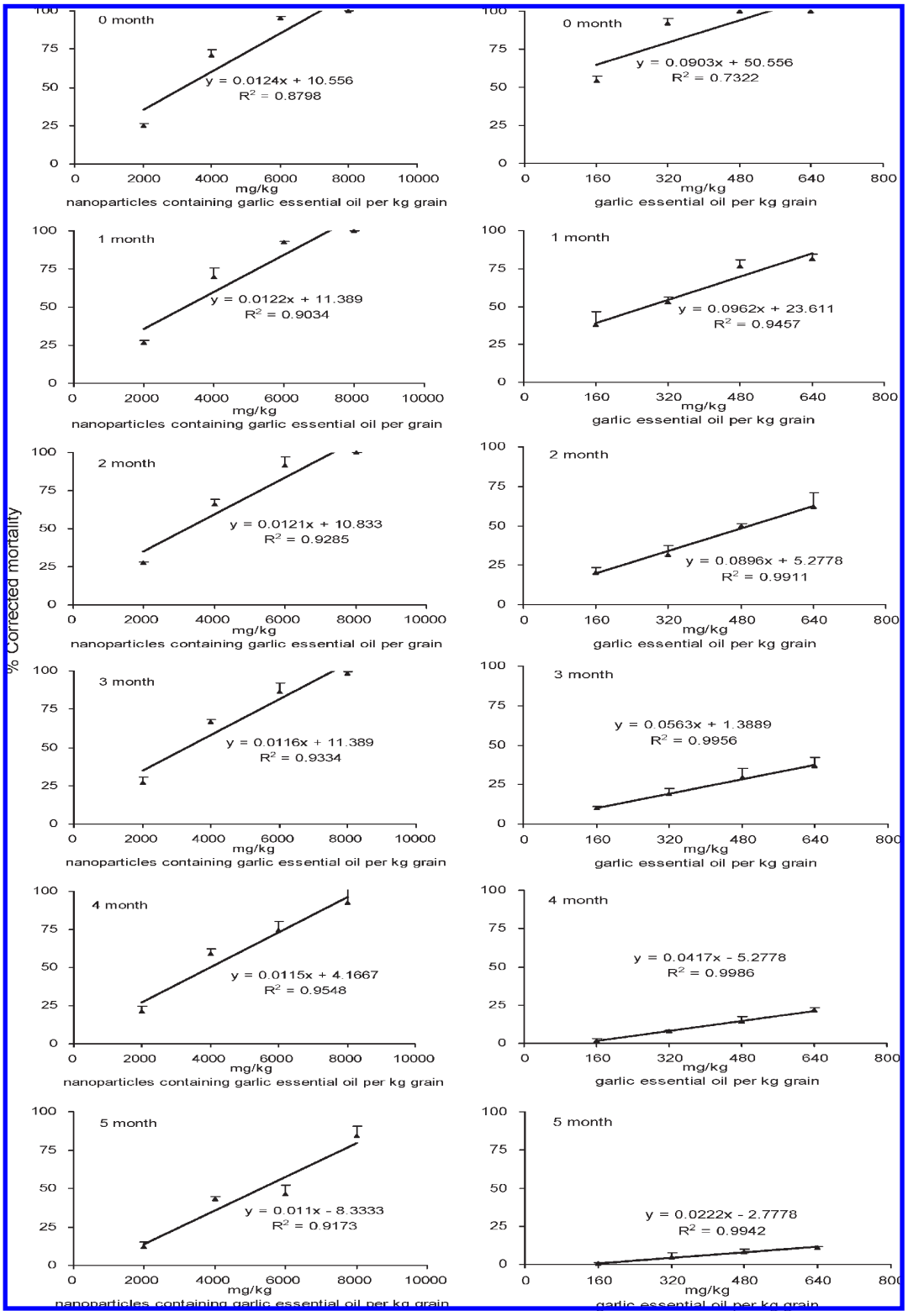

Figure 4. Corrected mortality of adult *T. castaneum* exposed for 5 d on rough rice treated with garlic essential oil pre/post-encapsulated, separately. Bioassays were conducted at monthly intervals. The solid line is the fitted linear regression line from the corrected mortality of adults. The small triangle represents the mean values ± SE (n = 3), while the error bars indicate the standard deviation of the means.

containing garlic essential oil are stable when stored for five months at 25 °C.

From the chromatographic analysis, results show that the amount of diallyl trisulfide decreased slowly with an extension of storage time (**Table 2**). At month 0, this study noted a slight decrease (4.6%), but the variance was not significant compared to the free garlic essential oil. After five months, the amount of diallyl trisulfide remaining in the nanoparticles compared to the free garlic essential oil decreased significantly (10.4%), but when compared to samples extracted immediately after encapsulation (0 month), the difference (6.1%) was not significant. The result likewise indicates that diallyl trisulfide is more stable in the nanoparticles than the substrate existing in free garlic essential oil or alone (*11*). This could effectively decrease the degradation

of diallyl trisulfide when faced with the characteristics of rapid volatility, which better preserves the biological properties of garlic oil, including cholesterol reduction, cancer prevention, and strong insecticidal activities against pest insects (*29*, *30*).

As for the second major constituent, diallyl disulfide, it was noted that the changing tendency of the content in nanoparticles was similar to that of diallyl trisulfide, but the speed of decrease was faster (**Table 2**). The content of diallyl disulfide remaining in the nanoparticles after 5 months was 23.1% less than free garlic essential oil, and 17.5% less than 0 month encapsulated oil, and these differences were both significant. The lower molecular weight relative to diallyl trisulfide is probably the factor responsible for this result. However, the content of diallyl sulfide after five months increased by 62.9% in the particles compared to the amount present in free garlic essential oil. The results could be attributed to the degradation of other sulfur-containing compounds (*1*).

Insecticidal Activities of Nanoparticles against Adult *T. castaneum*. The attack by more than 600 species of beetle pests on stored products of agricultural and animal origin causes quantitative and qualitative losses, as well as insect contamination in food commodities; hence, it is an important quality control problem that greatly concerns the food industry (*31*). In recent years, although many natural products from plants were proven to possess high insecticidal activities against stored-product pests (*32*), the effectiveness is limited by rapid volatilization (*10*).

In the present investigation, the insecticidal activity of garlic essential oil-loaded nanoparticles was evaluated against adult *T. castaneum* and compared to the free garlic essential oil treatment (**Figure 4**). Results show that doses and month were all significant for the mortality of test pests after five days of exposure when treated with encapsulated garlic oil and free garlic essential oil ($F = 2906.531$, df = 3, $P = 0.000$; $F = 205.848$, df = 5, $P = 0.000$; $F = 127.972$, df = 3, $P = 0.000$; and $F = 317.159$, df = 5, $P = 0.000$). Herein, concentration and month are the main effects, while corrected mortality is the dependent variable. The efficacy of garlic essential oil during pre- and post-encapsulation was positively related to dose and negatively related to time. The mortality of adults increased with increasing concentrations of garlic essential oil in groups treated with either free essential oil or with nanoparticles loaded with essential oil; however, the mortality of adults decreased gradually with the extension of the grain's storage time (**Figure 4**).

During the whole experiment, results show that there was a significant difference in the controlling efficacy performed by treatment groups treated with free garlic essential oil and those treated with nanoparticles loaded with essential oil. For the groups treated with free essential oil, the mortality decreased rapidly from month 0 to month 5, especially on the second month after storage of treated grain (**Figure 4**). The mortality of adults on the grain treated with 640 mg/kg was $< 40\%$ at the third month and only 11% by the fifth month, indicating that residues were becoming less and less active over time. However, for groups treated with nanoparticles, results show that the control efficacy decreased gradually with the extension of storage time of treated grain, but the speed of insecticidal loss became very slow, as seen in the regression coefficient of the linear regression model in **Figure 4**, which was 0.0124, 0.0122, 0.0121, 0.0116, 0.0115, and 0.0110, respectively. Moreover, the corrected mortality against test pests remained over 80% at 8000 mg/kg (equal to 640 mg/kg of free garlic essential oil) after month 5 (**Figure 4**). Based on the changing tendency of insecticidal activities from nanoparticles, insecticidal loss may be attributed to variations of major compositions of post-encapsulated garlic essential oil, which indirectly indicates that the toxicity of diallyl disulfide and diallyl trisulfide are stronger than diallyl sulfide if treated against adult *T. castaneum* (*30*).

Additionally, as seen from the results in **Figure 4**, the control efficacy of nanoparticles was lower relative to the free garlic essential oil at month 0, but this became gradually stronger in the following months compared to the free garlic essential oil, indicating that nanoparticles slowly and persistently releases active components.

Results of nanoparticle-encapsulated essential oil against stored-product pests differed from reports by Hyari et al. in 1977 (*33*), who did not detect any significant difference in residual efficacy between emulsifiable concentrates of malathion and fenitrothion versus microencapsulated products in bioassays with several store-product beetles. In the present test, the control efficacy of nanoparticles containing garlic oil was superior to that of free garlic essential oil; moreover, persistence could be maintained for five months at $> 80\%$ mortality at dose of 8000 mg/kg, which is equal to 640 mg/kg of free essential oil. Our findings indicate the feasibility of using nanoparticles loaded with garlic essential oil in controlling stored-product insect pests.

Additionally, with the nanosize property, nanoparticles may not affect the physical properties of grain such as bulk density and fluidity of grain when mixed with grain, by reducing the friction force between grain and nanoparticles. Meanwhile, with its water-soluble property, the particles could easily be washed off from the protectant using water. Thus, the formulation of garlic essential oil possessed potent application potentials in stored-product protection against stored-product pests when mixed directly with stored products.

However, further work is on the progeny production of treated adults, the insecticidal stability of nanoparticles to temperature and moisture content, and testing of mammalian toxicity, feasibility, and cost in applying in large-scale trials. Likewise, screening species of target insects and evaluating the effects on nutritional quality and residues prior to the commercial application of food commodities exposed to these nanoparticles require more in-depth studies.

ACKNOWLEDGMENT

This work was supported by National Facilities and Information Infrastructure for Science and Technology Program (2005DKA21105).

LITERATURE CITED

(1) Corzo-Martínez, M.; Corzo, N.; Villamiel, M. Biological properties of onions and garlic. *Trends Food Sci. Technol.* **2007**, *18*, 609–625.

(2) Namazi, H. The role of garlic in the prevention of ischemia-reperfusion injury: a new mechanism. *Mol. Nutr. Food Res.* **2008**, *52*, 739–740.

(3) Sfaxi, I. H.; Ferraro, D.; Fasano, E.; Pani, G.; Limam, F.; Marzouki, M. N. Inhibitory effects of a manganese superoxide dismutase isolated from garlic (*Allium sativum* L.) on in vitro tumoral cell growth. *Biotechnol. Prog.* **2009**, *25*, 257–264.

(4) Li, T.; Ito, K.; Sumi, S.; Fuwa, T.; Horie, T. Protective effect of aged garlic extract (AGE) on the apoptosis of intestinal epithelial cells caused by methotrexate. *Cancer Chemother. Pharmacol.* **2009**, *63*, 873–880.

(5) Park, I. K.; Choi, K. S.; Kim, D. H.; Choi, I. H.; Kim, L. S.; Bak, W. C.; Choi, J. W.; Shin, S. C. Fumigant activity of plant essential oils and components from horseradish (*Armoracia rusticana*), anise (*Pimpinella anisum*) and garlic (*Allium sativum*) oils against *Lycoriella ingenua* (Diptera: Sciaridae). *Pest Manage. Sci.* **2006**, *62*, 723–728.

(6) Park, I. K.; Shin, S. C. Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus* Kolbe). *J. Agric. Food Chem.* **2005**, *53*, 4388–4392.

(7) Kimbaris, A. C.; Kioulos, E.; Koliopoulos, G.; Polissiou, M. G.; Michaelakis, A. Coactivity of sulfide ingredients: a new perspective of the larvicidal activity of garlic essential oil against mosquitoes. *Pest Manage. Sci.* **2009**, *65*, 249–254.
(8) Ho, S. H.; Koh, L.; Ma, Y.; Huang, Y.; Sim, K. Y. The oil of garlic, *Allium sativum* L. (Amaryllidaceae), as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *Postharvest Biol. Technol.* **1996**, *9*, 41–48.
(9) Huang, Y.; Chen, S. X.; Ho, S. H. Bioactivities of methyl allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* **2000**, *93*, 537–543.
(10) Dayan, F. E.; Cantrell, C. L.; Duke, S. O. Natural products in crop protection. *Bioorg. Med. Chem.* **2009**, *17*, 4022–4034.
(11) Li, Y.; Xu, S. Y.; Sun, D. W. Preparation of garlic powder with high allicin content by using combined microwave-vacuum and vacuum drying as well as microencapsulation. *J. Food Eng.* **2007**, *83*, 76–83.
(12) Bertolini, A. C.; Siani, A. C.; Grosso, C. R. F. Stability of monoterpenes encapsulated in gum arabic by spray-drying. *J. Agric. Food Chem.* **2001**, *49*, 780–785.
(13) Latheeef, M. A. Influence of pyrethroid formulations and sprayer nozzle size on tobacco budworm mortality on cotton. *Southwest. Entomol.* **1995**, *20*, 49–54.
(14) Arthur, F. H. Evaluation of an encapsulated formulation of cyfluthrin to control *Sitophilus oryzae* (L.) on stored wheat. *J. Stored Prod. Res.* **1999**, *35*, 159–166.
(15) Sozer, N.; Kokini, J. L. Nanotechnology and its applications in the food sector. *Trends Biotechnol.* **2009**, *27*, 82–89.
(16) Pinto Reis, C.; Neufeld, R. J.; Ribeiro, A. J.; Veiga, F.; Nanoencapsulation, I. Methods for preparation of drug-loaded polymeric nanoparticles. *Nanomedicine* **2006**, *2*, 8–21.
(17) McClean, S.; Prosser, E.; Meehan, E.; O'Malley, D.; Clarke, N.; Ramtoola, Z.; Brayden, D. Binding and uptake of biodegradable poly-lactide micro and nanoparticles in intestinal epithelia. *Eur. J. Pharm. Sci.* **1998**, *6*, 153–163.
(18) Peng, J.; Li, J.; Li, Q. Preparation and application of water-soluble fragrance-loaded nano-dispersion capsules. *Flavour Fragrance Cosmet.* (*China*) **2008**, *3*, 22–25.
(19) Hu, Y.; Xie, J.; Tong, Y. W.; Wang, C. H. Effect of PEG conformation and particle size on the cellular uptake efficiency of nanoparticles with the HepG2 cells. *J. Controlled Release* **2007**, *118*, 7–17.
(20) Prego, C.; Torres, D.; Fernandez-Megia, E.; Novoa-Carballal, R.; Quinoá, E.; Alonso, M. J. Chitosan−PEG nanocapsules as new carriers for oral peptide delivery effect of chitosan pegylation degree. *J. Controlled Release* **2006**, *111*, 299–308.
(21) Vila, A.; Sánchez, A.; Tobío, M.; Calvo, P.; Alonso, M. J. Design of biodegradable particles for protein delivery. *J. Controlled Release* **2002**, *78*, 15–24.
(22) Tobío, M.; Sánchez, A.; Vila, A.; Soriano, I. I.; Evora, C.; Vila-Jato, J. L.; Alonso, M. J. The role of PEG on the stability in digestive fluids and in vivo fate of PEG-PLA nanoparticles following oral administration. *Colloids Surf., B* **2000**, *18*, 315–323.
(23) Shaikh, J.; BhosalE, R.; Singhal, R. Microencapsulation of black pepper oleoresin. *Food Chem.* **2006**, *94*, 105–110.
(24) Guo, H.; Zhao, X. Preparation of microcapsules with narrow-size distribution by complex coacervation: effect of sodium dodecyl sulphate concentration and agitation rate. *J. Microencapsulation* **2008**, *25*, 221–227.
(25) Zhu, A.; Pan, Y.; Dai, S.; Li, F.; Shen, J. Preparation of *N*-maleoylchitosan nanocapsules for loading and sustained release of felodipine. *Biomacromolecules* **2009**, *10*, 1997–2002.
(26) Katas, H.; Cevher, E.; Alpar, H. O. Preparation of polyethyleneimine incorporated poly(D,L-lactide-*co*-glycolide) nanoparticles by spontaneous emulsion diffusion method for small interfering RNA delivery. *Int. J. Pharm.* **2009**, *369*, 144–154.
(27) Zili, Z.; Sfar, S.; Fessi, H. Preparation and characterization of poly-ε-caprolactone nanoparticles containing griseofulvin. *Int. J. Pharm.* **2005**, *294*, 261–267.
(28) Clark, B. C.; Jones, B. B.; Jacobucci, J. A. Characterization of the hydroperoxides derived from singlet oxygen oxidation of (+)-limonene. *Tetrahedron Suppl.* **1981**, *37*, 405–409.
(29) Amagase, H. Clarifying the real bioactive constituents of garlic. *J. Nutr.* **2006**, *136*, 716–725.
(30) Amonkar, S. V.; Banerji, A. Isolation and characterization of larvicidal principle of garlic. *Science* **1971**, *174*, 1343–1344.
(31) Rajendran, S.; Sriranjini, V. Plant products as fumigants for stored-product insect control. *J. Stored Prod. Res.* **2008**, *44*, 126–135.
(32) Isman, M. B. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* **2006**, *51*, 45–66.
(33) Hyari, S.; Kadoum, A. M.; LaHue, D. W. Laboratory evaluations of emulsifiable and encapsulated formulations of malathion and fenitrothion on soft red winter wheat against attack by adults of four species of stored-product insects. *J. Econ. Entomol.* **1977**, *70*, 480–482.

Received July 5, 2009. Revised manuscript received September 14, 2009. Accepted September 15, 2009.