Characterization of Clove Oil Nanoparticles and Their Insecticidal Activity against *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae)

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ABSTRACT

The application of essential oils to storage pest control requires an appropriate formulation formed by biodegradable compounds. This formulation should protect essential oils from degradation and evaporation while simultaneously allowing sustained release. This study aims to characterize nanoparticles loaded by clove essential oil-based polymer polyethylene glycol (PEG) and to investigate their insecticidal activity. In this study, clove oil solid dispersion was prepared using the fusion or melting-dispersion method with polyethylene glycol 6000 (PEG 6000) to form a binary solid dispersion system. The ratio of 10% of clove oil to PEG was optimal and had low PDI, small size, and the highest clove oil loading efficiency. Their size changed from 179 to 197 nm after 24 weeks of storage and the loading efficiency decreased for about 36%. Clove oil formulation in nanoparticles could not enhance the toxicity of clove oil but yet still showed high contact toxicity to *C. ferrugineus*. These formulations also have the slow and persistent release of the bioactive.

INTRODUCTION

Rusty grain beetle, *Cryptolestes ferrugineus* (Steph.) has a wide distribution and damaging stored grains (Oehlschlager, Wong, Verigin, & Pierce Jr, 1983). This beetle is one of the most common pests in stored grain in the United States and Canada (Flinn & Hagstrum, 1998). Various protection methods and instruments against storage pests, including chemical, physical and biological control, environmental manipulation, and the use of resistant varieties, have been implemented individually or through the integrated control program (Lo, 1986). In the control of stored product pests, the synthetic insecticide is frequently used (Gasch, 2014). Alternative control can be carried out using essential oils and their components, where botanical insecticides have advantages over synthetic insecticides in terms of low mammalian toxicity, rapid degradation, and local availability.

Botanical insecticides are most suitable for the production of organic food in industrialized countries concerning the management of agricultural pests, but they can play a greater role in the production and post-harvest protection of the food in developing countries (Isman, 2006). Botanical pesticides are experiencing the awakening of biopesticides because they have many eco-toxicological advantages like minimizing human toxicity, rapid degradation, thereby reducing the environmental impact and making them suitable insecticides for organic farming (Cosimi, Rossi, Cioni, & Canale, 2009). To develop new control methods, several plant compounds have been tested against the targeted insect pest of the stored foods (Paranagama, Abeysekera, Nugaliyadde, & Abeywickrama, 2003; Regnault-Roger & Hamraoui, 1994).

The use of many aromatic plants as insecticides can be, among them cloves (*Syzygium aromaticum*). Cloves are tropical annual crops found in Indonesia. Clove essential oil is commonly used as a food flavoring and medicinal and cosmetic ingredients (Isman, 2006). The essential oil has been widely reported to be able to control various...
types of pests. Eugenol (2-methoxy-4-allylphenol) is a major component of clove oil (Isman, 2006; Jirovetz et al., 2006; Park & Shin, 2005). Eugenol showed a variable of specific LD50 values for pest species (Koul, Walia, & Dhaliwal, 2008). So the essential oil of cloves has the potential to be developed as a botanical pesticide.

The marketing challenges for essential oil-based pesticides include the consistency of the oil in the storage, transport of active ingredients, and the impact of the residual active ingredients after application (Isman, 2016). Some major obstacles in the utilization of botanical pesticides are poor water solubility and high degradation rates of many botanical pesticides (da Costa et al., 2014; Moretti, Sanna-Passino, Demontis, & Bazzoni, 2002) since pesticides must long persistence to control pests. One of the research topics that require attention to overcome these obstacles is the use of nanotechnology. The application of essential oils requires an appropriate formulation formed by biodegradable compounds that protect essential oils from degradation and evaporation while simultaneously allowing sustained release through nanoformulation (Martín, Varona, Navarrete, & Cocero, 2014).

The commonly used material in the encapsulation of botanical pesticides is the polymer. The polymer nanoparticles are the most promising for aromatic oil nanoformulations (Werdin González, Stefanazzi, Murray, Ferrero, & Fernández Band, 2015). The main advantages of using polymers in the manufacture of nanoparticles are several types of water-soluble, non-toxic, and generally applicable polymers for various materials (Chiou & Riegelman, 1971). The interaction of nanoparticles with organisms depends heavily on the surface aspects of the material, which can be explained by their chemical or hydrophilic and hydrophobic properties (Gupta & Curtis, 2004). Some water-soluble polymers that are commonly used in nanotechnologies are polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), chitosan, sodium alginate, and gelatin. There are several methods that can be used in the manufacture of polymer nanoparticles, such as the simple method of solid dispersion.

This study aimed to formulate a new system for botanical insecticides by nanoparticles loaded with clove essential oil-based polymer PEG, to characterize the clove oil nanoparticles, and to investigate their insecticidal activity against C. ferrugineus. In this study, the clove essential oil nanoparticles were made by using the fusion method of the melting-dispersion method with polyethylene glycol 6000 (PEG 6000) to form a binary solid dispersion system. Characterization was performed by using a transmission electron microscope (TEM), UV visible spectrophotometer, dynamic light scattering particle size analyzer, and gas chromatography-mass spectrometry (GC-MS). The bioactivity was then tested on C. ferrugineus storage pest.

**MATERIALS AND METHODS**

The research was conducted from December 2017 to May 2019 at the Toxicology Laboratory and Pest Laboratory of Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Brawijaya, Malang; Central Laboratory of Minerals and Advanced Materials, Faculty of Mathematics and Natural Science, State University of Malang; Laboratory of Production Unit, Chemical Engineering Department, State Polytechnic of Malang, and Laboratory of Material Physic, Department of Physics, Institute of Technology Sepuluh Nopember Surabaya.

**Insects Culture**

The 1-14 days old C. ferrugineus adults were obtained from the laboratory of pest, Faculty of Agriculture, Universitas Brawijaya, Malang.

**Preparation of Clove Oil Nanoparticles**

The main ingredients in the preparation of this nanoparticle were clove essential oil obtained from commercial companies and PEG 6000. Clove oil nanoparticles were prepared by the method of melting dispersion with some modifications (Yang, Li, Zhu, & Lei, 2009). Some PEG 6000 was heated at 65°C (100 g per part). After melting, PEG was blended with 2.5, 5.0, 7.5, 10.0 g clove oil. The solution was stirred for 30 minutes. The solution was then naturally cooled for 12 hours at 12°C, grounded in a cooled mortar (cooled at 12°C), and sifted using a 200 mesh sieve. The nanoparticles were stored at 27±2°C to further testing.

**Loading Efficiency of Clove Oil in Nanoparticles**

The nanoparticles were diluted with absolute ethanol-H2O (75:25) and heated for 30 minutes at 50°C. The absorbance of each concentration was tested using a UV visible spectrophotometer
[Shimadzu, Shimadzu Corp, Kyoto, Japan] at 285.5 nm. After 5 days of storage, 0.1 g per part of clove oil nanoparticles was dissolved in 2 ml of absolute ethanol-H$_2$O (75:25) and heated at 50°C for 30 minutes. At 285.5 nm, the absorption of the solution was determined and the values are compared to the standard curve. Each analysis was repeated three times.

**Size of Clove Oil Nanoparticles**

Dynamic Light Scattering (DLS) using Malvern Zetasizer NanoS (Malvern Instruments, UK) has determined the average size and the distribution. After 3 days of storage, at 10 ml distilled water, 0.2 g clove nanoparticle samples were suspended. Then using filter paper Wathman No. 1, the suspension was filtered. Each analysis was repeated three times.

**Clove Oil Composition**

The composition of clove oil in pre/post-nano formulations was determined by the Shimadzu Model QP-2010 Gas Chromatography-Mass Spectrometer (GCMS). The 0.5 g of 10% clove oil nanoparticles were dissolved in 5 ml of distilled water for oil extraction. After heated for 30 minutes at 50°C, 4 ml of absolute ether was then added. Based on a comparison of the retention times with the computer matching spectral MS data against Wiley 8, the components were identified.

**Residual Contact Toxicity**

The 20 g of rice samples were mixed with only clove oil or 10% clove oil nanoparticles. Concentrations of clove oil ranged from 0.05% to 0.25% and nanoparticles of clove oil ranged from 0.6% to 3% (w/w) (same concentration used to calculate clove oil loading efficiency). For oil treatment, the rice samples were mixed with 3 ml of the hexane clove oil solution and air-dried for 2 hours. The rice samples were then, put in containers of 100 ml glass and sealed. Clove oil or the nanoparticles were mixed with rice and vigorously shaken to spread the compound. The controls used were rice samples mixed with hexane or PEG 6000 alone. The test was carried out periodically until 16 weeks. For each period, 20 insects are placed into a glass container. Replication was carried out four times.

**Statistical Analysis**

The percentage of corrected mortality was calculated based on Abbot (1925). Using the statistical program SPSS 13.0, the mortality data at 72 hours were subjected to probit analysis to obtain the LC50 value. Analysis of Variance (ANOVA) and Duncan's Multiple Range Test was used to perform the mean comparisons.

**RESULTS AND DISCUSSION**

In this research, clove essential oil nanoparticles were produced using a solid dispersion strategy with a melting method using polyethylene glycol (PEG) to form a binary system. The method is very simple and easy compared to other nanotechnologies. Furthermore, during the melt-dispersion encapsulation process, the temperature was set much lower procedure to protect the essential oil constituents from high volatilization, especially for components with a low boiling point. In addition, the preparation period for PEG coating nanoparticles loaded with clove oil was very short. The simple procedure and short preparation time made it easier for nanoparticles containing essential oil to be generated (Yang, Li, Zhu, & Lei, 2009). This method is very valuable for low dose compounds because the maximum concentration is in the range of 5 to 10% (w/w) (Chiou & Riegelman, 1971).

The nanoformulation of clove oil into a solid dispersion by PEG 6000 can be stable by preventing them from rapid volatilization and degradation. To increase its bioavailability, the amount of the bioactive containment on nanoformulation may be reduced (Werdin González, Gutiérrez, Ferrero, & Fernández Band, 2014). Since relative humidity is the important variable for determining the quality of the grain, solid clove oil nanoformulation is desirable for the control of stored pests.

**Characterization of Nanoparticles**

The size, polydispersity index (PDI), and loading efficiency of clove oil nanoparticles were presented in Table 1. The PDI is an indicator of nanoparticles' size distribution. When PDI values are lower than 0.3, these indicated that the distribution of nanoparticles is very narrow (Werdin González, Gutiérrez, Ferrero, & Fernández Band, 2014). Another important variable for nanoparticles is oil loading efficiency. A high loading efficiency means that the nanoparticles are good. In this experiment, the efficiency of loading clove oil was positively associated with the quantity of oil, i.e., it increased with the ratio increment of clove oil to PEG. These results suggest that the method of melt-dispersion
for the preparation of nanoparticles of PEG-coated clove essential oil is feasible, with a 76% high loading efficiency and 10% ideal ratio of oil to PEG.

The best relationship between the 3 variables evaluated was the 10% clove oil-PEG ratio; i.e., it had a low PDI (<0.3), a small size, and the highest efficiency of loading clove oil. While the smallest size and the lowest PDI were found in 2.5% of clove oil nanoparticles. These nanoparticles also had the lowest loading efficiency. Based on the results, the 10% clove oil nanoparticles were chosen to characterize the size and composition during 24 weeks of storage and to evaluate the residual contact toxicity.

Nanoparticles have a PDI of 0.22-0.31 which indicates that nanoparticles are moderate polydisperse systems. The analysis of PDI results used approximate values for dispersity parameters (Nobbbmann, 2017). Moderate polydisperse systems tend to be stable compared to broad polydisperse systems because broad polydispersity tends to form aggregates. The water-soluble synthetic polymers based on oxyethylene are polyethylene glycols (PEGs) (Craig, 1995). This polymer is commonly used because of its high hydrophilicity and ability to improve the biocompatibility of nanoparticles (Gupta & Curtis, 2004). They also have good solubility in many organic solvents, low melting point (below 65°C), low toxicity, ability to dissolve some compounds, and could improve compound moisture (Hu, Xie, Tong, & Wang, 2007; Koh, Chuah, Talekar, Gorajana, & Garg, 2013). Polyethylene glycol is estimated to be one of the ideal universal carriers for the most poorly soluble chemicals (Chiou & Riegelman, 1971). In addition, PEG can also increase the gastrointestinal fluid stability of the nanocapsules and improve sustained intestinal absorption (Prego et al., 2006; Tobio et al., 2000; Vila, Sánchez, Tobio, Calvo, & Alonso, 2002).

Table 1. Average size, polydispersity index (PDI), and loading efficiency of clove oil nanoparticles

| Percentage of clove oil (w/w%) | Average size (nm) | PDI  | Loading efficiency (%) |
|---------------------------------|-------------------|------|------------------------|
| 2.5                             | 159.25            | 0.22 | 48.06                  |
| 5                               | 181.45            | 0.27 | 54.85                  |
| 7.5                             | 240.70            | 0.31 | 71.92                  |
| 10                              | 176.95            | 0.24 | 76.36                  |

In tablet or solid formulations, PEGs are well known as excipients. PEGs are considered to be inert in solid dosage forms and have very few interactions with other materials (Majumdar, Alexander, & Riga, 2010). One of the most promising methods for enhancing the bioavailability of materials such as plant-based insecticides that are difficult to dissolve in water is solid dispersion using PEG. Bioavailability can be greatly improved by reducing particle size to an absolute minimum and increasing the wettability of the material. Typically, they are described as amorphous materials, primarily obtained by two methods, such as solvent melting and evaporation (Vasconcelos, Sarmento, & Costa, 2007).

In this study, the melting technique is the method used. The rapid cooling of a liquid below its melting point (Tm) will result in an amorphous state with a liquid’s structural features, but with a much higher viscosity (Hancock & Zografi, 1997). Material (such as essential oil) or rapid cooling of melted PEG can act as a crystallization inhibitor resulting in a higher percentage of amorphous and imperfectly crystalline material (Werdin González, Gutiérrez, Ferrero, & Fernández Band, 2014). Amorphous characterizes polymeric molecules widely used as excipients. The potential for crystallization during handling and storage is still present, as molecules in the amorphous state are thermodynamically metastable compared to the crystalline state (Hancock & Zografi, 1997).

PEG has a helical conformation consisting of seven chemical units and two turns in a 19.3 Å fibre recognition cycle and a 20 + 1.3 Å sequence of seven units in length (Craig, 1995). PEG chains are orientated at random in the molten state. Nucleation can occur through either homogeneous or heterogeneous mechanisms when the melt cools (Chidavaenzi, Buckton, & Koosha, 2001). Diffusive transport processes are typically slightly faster and isotropic than in crystal form because of the higher free volume of amorphous materials (Hancock & Zografi, 1997). Similar to other nanoencapsulation, this may clarify clove oil nanoparticles release profile in these experiments (Werdin González, Gutiérrez, Ferrero, & Fernández Band, 2014; Yang, Li, Zhu, & Lei, 2009). The oil-loading efficiency of the nanoparticle could exceed 76% in this study at the optimum ratio of clove oil to PEG (10%). The oil loading efficiency could also reach 80% with the
optimum ratio of garlic oil to PEG (10%) (Yang, Li, Zhu, & Lei, 2009). The dynamic scattering particle analyzer was used to investigate the average particle size. Within 6 months of storage periods, the average particle size increased from 179 to 197 nm (Table 2). According to these findings, an agglomeration mechanism occurring during storage is likely, promoting an increase in the size of NP, which correlates with PDI. During this time, the number of EOs decreased from 76 to 40% (Fig. 1). Because of the strong hydrogen bond between PEG and water, PEG possibly caused the more condensed chemical suspensions to crystalize slowly (Chidavaenzi, Buckton, & Koosh, 2001). Physically and chemically, amorphous solids are typically less stable than the corresponding crystals (Yu, 2001).

Table 2. Clove oil nanoparticle (NP) size and polydispersity index (PDI) during 6 months of storage

| Storage Time (weeks) | NP size (nm) ± SE | PDI       |
|----------------------|------------------|-----------|
| 1                    | 179 ± 1.69 a     | 0.236 ± 0.007 |
| 8                    | 189 ± 1.41 b     | 0.266 ± 0.012 |
| 16                   | 198 ± 1.68 c     | 0.291 ± 0.022 |
| 24                   | 197 ± 0.61 c     | 0.202 ± 0.005 |

Remarks: Means followed by the same letter were not statistically different using the LSD test (p<0.05)

Fig. 1. Loading efficiency of clove oil nanoparticles during 24 weeks of storage
There was also no issue found with the clove oil nanoparticles when the clove oil nanoparticles were dissolved in distilled water. Its greater solubility is one of the key advantages of this type of nanoparticle formulation. This may be a result of the amorphous state of nano-sized particles and the PEG reached during the process of nanoparticle formation. Standard processes may create amorphous solids, which are the common type of unstable substance materials that look like polymers (Yu, 2001). During different processes, the amorphous form can occur, which may affect the dissolution rate and transport characteristics of the material (Vippagunta, Brittain, & Grant, 2001). In addition, many excipients are completely or partially amorphous to the formulator (e.g., microcrystalline cellulose, starch, poly(vinylpyrrolidone), and other excipients may be intentionally made amorphous in order to increase functionality (Hancock & Zografi, 1997).

**Chemical Composition of Clove Oil Pre and Post-Nanoformulation**

In this investigation, we obtained clove oil by water distillation. The pre-/post-formulation qualitative analyses of clove oil were performed using GC-MS during the 6 months storage period (Table 3). This clove oil comprises in total of 3 identified constituents. There are two main components at pre-nanoformulation, considered to be eugenol and Trans-Caryophyllene. Results are consistent with previous reports regarding the content of the clove oil. Clove terpenes are present in the higher boiling fraction of the clove oil, which can be obtained by H2O distillation (Zheng, Kenney, & Lam, 1992). The essential oil obtained by steam distillation of clove leaves consists mainly of eugenol and many other terpenoids (Dayan, Cantrell, & Duke, 2009). A major component of clove oil is Eugenol (2-methoxy-4-allylphenol) (Isman, 2006; Jirovetz et al., 2006; Park & Shin, 2005). Commonly, clove oil consists of more than 92% eugenol and also contains eugenyl acetate, b-caryophyllene (Kubeczka & Formácek, 2002), and smaller quantities of other elements, such as benzyl alcohol (Chaieb et al., 2007).

In this study, it was found that PEG 6000 can stabilize clove oil in a polymer that enables it to reduce volatility. Moreover, we also observed that during the storage process, the chemical composition of clove oil in nanoformulation was unchanged. In other nanoformulation used PEG 6000, there are no oxidized or hydrolytic derivatives were found from the original composition (Werdin González, Gutiérrez, Ferrero, & Fernández Band, 2014). These indicated that there was no major component damage occurred during the storage period.

The main component of clove oil before the formulation is eugenol and this compound is maintained after post formulation until 24 weeks storage. Eugenol has been reported to affect the octopamine receptor (neuromodulator) specifically and to exert its insecticidal properties through this action (Price & Berry, 2006). Eugenol is a fast-acting contact insecticide that acts on a wide range of arthropod pests and is also used on many ornamental plant pests, such as, mites, aphids, thrips, and armyworms (Dayan, Cantrell, & Duke, 2009). Trans-caryophyllene with a relatively small amount was another key compound of clove oil and its quantity was not detected after the nanoformulation process. In toxic compounds of terpenoids, however, eugenol has greater contact toxicity to S. granarius rather than caryophyllene (Plata-Rueda et al., 2018).

**Table 3. Pre-/post-nanoformulation of the chemical composition of clove and percentage content of each compound during 24 weeks of storage**

| Retention Time (minute) | Compound          | Pre-formulation (%) | Post formulation | 1 week | 8 weeks | 12 weeks | 16 weeks | 24 weeks |
|-------------------------|-------------------|---------------------|------------------|--------|---------|----------|----------|----------|
| 3.03                    | Methylbutanal     | -                   | -                | 8.02   | 12.04   | -        | -        | -        |
| 23.29                   | Eugenol           | 92.43               | 100              | 91.98  | 87.96   | 100      |          |          |
| 25.28                   | Trans-Caryophyllene| 7.57                | -                | -      | -       | -        | -        | -        |
Residual Contact Toxicity of Clove Oil and Clove Oil Nanoparticle

More than 600 species of beetle pests were reported to attack stored agricultural products and causing quantitative and qualitative losses. Aside from the stated matter, insect contamination in food commodities is also a major quality control issue of concern to the food industry (Rajendran & Sriranjini, 2008). In organic agriculture, the use of essential oils for pesticides seems promising, but all these natural insecticides work very rapidly and their efficacy is limited by the fact that they are most likely to volatilize very quickly (Dayan, Cantrell, & Duke, 2009). In this study, the clove oil nanoparticles were used to control adult C. ferrugineus, and these were compared to the free clove essential oil treatment. Clove essential oils have been widely reported to control various types of pests. Clove oil is also effective against other storage pests such as Tribolium castaneum, S. zeamais (Ho, Cheng, Sim, & Tan, 1994), and A. obtectus in storage facilities (Viteri Jumbo, Faroni, Oliveira, Pimentel, & Silva, 2014). There was no T. castaneum offspring that emerged from rice treated with non-polar clove extract (Ho, Cheng, Sim, & Tan, 1994).

The nanoparticles can exhibit different properties compared with their bulk counterparts (Anjali et al., 2010). In this experiment, the results showed no increase in the contact toxicity of clove oil nanoparticles compared to clove oil for 16 weeks of storage. The toxicity of clove oil from the lowest to highest concentrations caused 100% mortality after 24 hours of exposure almost in all storage periods. Only at 3, 4 and 8 weeks of storage did not occur 100% of all deaths. This result is not in line with the reports of Werdin González, Gutiérrez, Ferrero, & Fernández Band (2014) and Yang, Li, Zhu, & Lei (2009), using the same nanoparticle system but different kind of oils and pest species, there was a strong relationship between concentrations and the increase of contact toxicity. However, the results were similar to Hyari, Kadoum, & Lahue (1977) that showed no significant differences in relative effectiveness between the encapsulated and emulsifiable formulations of malathion and fenitrothion for several storage pests (Hyari, Kadoum, & Lahue, 1977). The clove nanoparticle toxicity result against C. ferrugineus also similar to Ikawati, Himawan, Abadi & Tarno (2020) where the nanoformulation could not enhance clove oil contact toxicity to T. castaneum. The absence of increment in the contact toxicity, when compared with free garlic oil, was probably due to the main absorption route. The absorption routes were not only direct contact with the cuticle but also from the many absorption routes which were involved together, such as respiratory pathway as fumigants. There is also a possibility of loss or reduction of other properties such as repellent and antifeedant properties. Changes in the insecticidal activity of nanoparticles, such as a decrease in insecticide properties also could be attributed to variations in the main composition of post-encapsulated essential oils (Yang, Li, Zhu, & Lei, 2009).

This result indicates that the toxicity combination of eugenol and trans-caryophyllene are stronger than individual compound against adult C. ferrugineus. The toxicity of the component mixture indicates a synergistic effect between the components, with all the components needed for the maximum toxicity of natural oils (Hummelbrunner & Isman, 2001; Jiang, Akhtar, Bradbury, Zhang, & Isman, 2009). There are synergistic effects of three types of oils containing eugenol, α-terpineol, and cinnamic alcohol, where the octopaminergic system mediates the activity of eugenol.

The residual effects of clove oil nanoparticles to C. ferrugineus at 2.4% and 3% concentrations are illustrated in Fig. 2 (data mortality from 120 hours exposure). Clove oil nanoparticles resulted in contact toxicity at these two concentrations over 16 weeks. After 16 weeks, the nanoformulation still produced more than 60% and 90%. However, for the nanoparticles treated group, the contact toxicity decreased gradually in line with the longer storage period. The rate of insecticide loss was low and this implies that the active component of nanoparticles still slowly and continuously be released.
Fig. 2. Residual contact toxicity of clove oil nanoparticles at 2.4% concentration (equal to 0.20% clove oil) and 3.0% concentration (equal to 0.25% clove oil) after 120 hours exposure against adults of *C. ferrugineus*.

Fig. 3. LC$_{50}$ values of clove oil nanoparticles for adults of *C. ferrugineus* (data mortality after exposure of 72 and 120 hours).
Values of LC\textsubscript{50} for adult *C. ferugineus* obtained from contact toxicity data after 3 and 5 days exposures are presented in Fig. 3. Under free clove oil treatments, 100% mortality occurred in all concentrations during all storage periods. Consequently, the LC\textsubscript{50} value was not able to be calculated. These conditions implied that the LC\textsubscript{50} value of clove oil is smaller than the clove oil nanoparticles. At the storage period of 3, 4, and 8 weeks, not all the insect pest were ceased. Thus, the LC\textsubscript{50} MC value at 0, 1, 2, 8, 12, and 16-week storage is smaller than the clove oil nanoparticles. At the storage period of 3, 4, and 8 weeks, the LC\textsubscript{50} values of clove oil were analyzed and revealed 0.044, 0.07, and 0.198%, respectively. Based on the LC\textsubscript{50} at the 3 weeks of storage, the toxicity of clove oil was better than nanoparticles (0.076%). After 4 and 8 weeks, however, the toxicity of nanoparticles (0.045 and 0.048%) was better than clove oil. The toxicity of clove oil nanoparticles was time-dependent. It decreased during the experiment, which was indicated by the increasing value of LC\textsubscript{50}. Moreover, with the nanosize property, the physical properties of grains may not be influenced by nanoparticles, when they were combined, since the friction between grains and nanoparticles would be minimized. Meanwhile, with its water-soluble characteristic, the substance would be easy to wash with water. Thus, clove oil formulation has the potential to be applied in the protection of stored products against storage pests by directly mixing with stored products.

In fumigant form, clove oil was able to cause 100% mortality after 24 hours of exposure compared to clove oil nanoparticles which did not cause any effect after 120 hours of exposure. This may suggest that nanoformulation decreases the volatility of clove oil constituents. This result is similar to other nanoparticles. In PEG nanoparticles containing geranium or bergamot essential oil, the coating also reducing fumigant properties (Werdin González, Gutiérrez, Ferrero, & Fernández Band, 2014). In solid lipid nanoparticles of *Artemisia arborescens* EO, compared with reference emulsions, nanoparticles have been able to minimize the rapid evaporation of essential oil (Lai, Wissing, Müller, & Fadda, 2006).

Polymer generally acts as an inert carrier that dominates the physical properties: solubility, the permeability of the membrane, and biodistribution (Zalipsky, 1995). The mode of action of nanoparticles in this study such as the mechanism of penetration and bioavailability of nanoparticles in the body of insects is still poorly understood. Several hypotheses could be carried out about the toxicological processes involved in the biological activity of nanoparticles, i.e. the penetration patterns, bioavailability of nanoparticles, and the detoxification mechanisms involved (Werdin González, Gutiérrez, Ferrero, & Fernández Band, 2014).

By PEG conjugation, the toxicity of a chemical may also be minimized. The chemical may be released in vivo or not, depending on the linkage between the components of the conjugate. Biological activity is responsible for the covalent bond substrate (Zalipsky, 1995). Dittmann (1973) reviewed the following parameters to be compared with three homologous series of alkyl polyglycol ethers: surface anesthesia, endoanesthesia, hemolysis, lethality, and reduction of surface tension. The various properties of PEG-alkyl ethers were largely dependent on the non-PEG portion, while the impact of the polyglycol chain was relatively small (Dittmann, 1973). In the other study, the polymeric doxorubicin prodrug experiment was used to link monomethoxy poly(ethylene glycol) and increased stability of the conjugates to alkaline degradation compared with free doxorubicin.

On the other hand, with regard to the free drug, derivatization was followed by a substantial decrease in toxicity in mice. Doxorubicin was not delivered by chymotrypsin incubation or in plasma form conjugates (Caliceti et al., 1993). The development of predictive relationships between structures and activities that are determined by the properties of nanoscale materials such as size, shape, surface chemistry, roughness, and surface coating (Nel et al., 2009). Further work is needed on the stability of the nanoparticle insecticide against temperature, humidity, UV rays, and other physical factors. Besides that, also needed biological efficacy to other species of pest and evaluate the applying cost in large-scale trials.

**CONCLUSION**

At the 10% optimum ratio of clove oil to PEG, the clove oil nanoparticles showed an average diameter < 180 nm, PDI < 0.250, and approximately 76% loading efficiency. Their size is considered stable after 6 months of storage and the loading
efficiency decreased by approximately 36%. In addition, the percentage of major component content did not indicate any difference between the nanoparticles and the clove oil. Clove oil formulation in nanoparticles caused high contact toxicity to *C. ferrugineus* also have the slow and persistent release of the bioactive.

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