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# Toxicity of Clove Oil Nanoparticles against Diamondback Moth *Plutella xylostella* (L.)

(Ketoksikan Nanozarah Minyak Cengkih terhadap Rama-rama Berlian *Plutella xylostella* (L.))

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ABSTRACT

*Plutella xylostella*, diamondback moth (DBM), has been one of the most challenging insects to control in the world to date. Environmentally friendly methods of control, such as the use of botanical insecticides, are available. A formulation that can sustain the main compound's level is required, which can be accomplished through soluble powder nanoformulation. The goal of this research was to test and evaluate the ability of clove oil nanoparticles produced from polyethylene glycol 6000 (PEG 6000) to control DBM utilizing a solid dispersion technique. Bioassay by leaf dip method in laboratory was used to test the lethal effect of clove oil nanoparticles (CO-NPs) on DBM. Clove oil nanoformulation was successful because it produced nanoparticles (179.98 nm in diameter) while maintaining high levels of the active component eugenol. Clove oil nanoparticles may increase clove oil's toxicity to DBM, seen from the $LC_{50}$ value after 24 h of treatment. The $LC_{50}$ values for clove oil nanoparticles after 24 and 48 h of treatment were 10.308 and 9.451%, respectively.

Keywords: Botanical pesticides; clove oil; nanoformulation; *Plutella xylostella*; solid dispersion

ABSTRAK

*Plutella xylostella*, rama-rama berlian (DBM), telah menjadi salah satu serangga yang paling mencabar untuk dikawal di dunia setakat ini. Kaedah kawalan mesra alam, seperti penggunaan racun serangga botani, tersedia. Formulasi yang boleh mengekalkan tahap sebatian utama diperlukan, yang boleh dicapai melalui nanoformulasi pepejal. Matlamat penyelidikan ini adalah untuk menguji dan menilai keupayaan nanozarah minyak cengkih yang dihasilkan daripada polietilena glikol 6000 (PEG 6000) mengawal DBM menggunakan teknik penyebaran pepejal. Bioassay melalui kaedah celup daun di makmal digunakan untuk menguji kesan maut nanozarah minyak cengkih (CO-NPs) pada DBM. Nanoformulasi minyak cengkih berjaya kerana ia menghasilkan nanozarah (diameter 179.98 nm) sambil mengekalkan tahap tinggi komponen aktif eugenol. Nanozarah minyak cengkih boleh meningkatkan ketoksikan minyak cengkih kepada DBM, dilihat daripada nilai $LC_{50}$ selepas 24 jam rawatan. Nilai $LC_{50}$ untuk nanozarah minyak cengkih selepas 24 dan 48 jam rawatan masing-masing adalah 10.308 dan 9.451%.

Kata kunci: Formulasi nano; minyak cengkih; penyebaran pepejal; *Plutella xylostella*; racun serangga botani

## INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* is a widespread and commercially significant pest of cruciferous crops (Xia et al. 2018). The DBM has become one of the most difficult insects to control in the world due to its biological and ecological traits, as well as its wide host range, which includes many plants with high aesthetic requirements (Talekar et al. 1993). Cabbage is one of the hosts that has been targeted, which could lead to crop failure in this plant. Synthetic pesticides are still employed to control pests, which can have a number of negative implications, including the development of resistance, resurgence, influence on natural pest foes, and contamination of the environment. DBM, on the other hand, has evolved resistance to the majority of insecticides now in use (Li et al. 2018). Basic integrated pest management cuts insecticide use, lowers negative environmental effects, and saves hundreds of millions of dollars each year (Zalucki et al. 2012). Botanical pesticides are a type of alternative pest control that can be used in conjunction with other pest control methods as part of an integrated pest management plan. These compounds' extensive range of pesticide activity can also help to reduce resistance selection in pest populations (Oliveira et al. 2019).

Indonesia has the potential to develop botanical insecticides since many plants, such as cloves (*Eugenia caryophyllata* Thunb), generate essential oils. Cloves are native plants that are widely farmed throughout Indonesia. Clove essential oil can be used to repel a range of pests. The biological activity has been studied for use against a variety of insect pests, and it has insecticidal properties (Kafle & Shih 2013). Aside from chemical considerations, commercialization necessitates a number of practical concerns, including regulatory requirements, the price of the oil commodity utilized as an active ingredient, the availability of large quantities of oil, and the chemical consistency of the oil (Isman 2016). One of the criteria for successful and efficient pesticide application is a proper formulation. It is difficult to create environmentally friendly, high-efficacy pesticide formulations from pesticide ingredients that are poorly water soluble and photosensitive. Weaknesses of existing formulations (emulsifiable concentrate, wettable powder, and emulsion in water) is to require an organic solvent and limited effectiveness (Cui et al. 2019). Two of the most major hurdles to the application of botanical pesticides are their poor water solubility and rapid degradation rates (da Costa et al. 2014), pesticides, on the other hand, are expected to last long enough to control pests. One solution to this problem is to use soluble powder nanoformulations. This technology has the potential to reduce indiscriminate pesticide spraying while simultaneously ensuring their safety (Nuruzzaman et al. 2016).

Botanical compounds mixed with nano-scale formulations have the potential to increase efficacy for long-term use in integrated pest management in agriculture (Oliveira et al. 2019). Pesticide formulations with nanoencapsulated pesticides can reduce pesticide dosage and human exposure, resulting in more ecologically friendly crop protection (Nuruzzaman et al. 2016). A solid dispersion is a collection of solid materials made up of at least two separate components, usually a hydrophilic and a hydrophobic materials (Das et al. 2012). As a carrier or matrix, a polymer might be employed. Monomers, which are repeating structural units that are joined together to form an extended structural framework, make up polymers (Baghel et al. 2016). to assess the lethal effects of clove oil (CO) and clove oil nanoparticles (CO-NPs) on DBM, so that can find out the potential of PEG-based clove oil nanoparticles created utilizing a solid dispersion approach to control DBM as a botanical pesticide.

## Materials and Methods

### CLOVE OIL NANOPARTICLES (CO-NPS) CHARACTERIZATION

*The CO-NPs preparation* A modified dispersion melting procedure was used to make the CO-NPs material (Yang et al. 2009). A total of 50 g of PEG 6000 was melted at 65 °C in a hotplate magnetic stirrer, followed by 5 cc of clove oil (obtained from commercial company), so that the concentration of clove oil used is 10%. To ensure that the PEG 6000 and clove oil were evenly incorporated, the solution was stirred for 30 min. The solution was then cooled at temperature 12 °C for 24 h to produce nanoparticles. In a mortar, the solids were crushed and sieved by a sieve (230 mesh).

*The size and dispersion of CO-NPs* A dynamic light scattering particle size analyzer (PSA-DLS, Malvern Instruments Zetasizer Nano-S) at 25 °C used to determine the mean size and size distribution. For 30 min, the 0.2 g of CO-NPs sample was dissolved in 10 mL distilled water. The solution was then filtered using Whatman No. 1 filter paper. The tests were repeated three times in total. The polidispersion index (PDI) data was analyzed using approximate values for the dispersion parameters (Nobbmann 2014). Monodisperse uniform (0.0), monodisperse narrow (0.0-0.1), polydisperse moderate (0.1-0.4), and polydisperse broad (>0.4) were the PDI parameters.

*The clove oil (CO) content* The chemical content of oil in the before and after nanoformulation was analyzed using gas chromatography-mass spectrometry (GC-MS Shimadzu Model QP-2010). To extract oil, 0.5 g of sample was diluted in 5 mL distilled water and for 30 min heated at 50 °C; the 4 mL pure ether was added to collect the extracted CO.

The chemicals were identified by comparing their retention periods and mass spectra to those in the library (comparison quality >80%). A Shimadzu Model QP-2010 Plus Mass Spectrometer with a HP-5 capillary column capillary column and a GCMS Shimadzu Model QP-

2010 Plus Mass Spectrometer were used for the GC-MS analysis. The carrier gas is helium, which has a flow rate of 1 mL min$^{-1}$.

### CLOVE OIL NANOPARTICLES (CO-NPS) TOXICITY

Toxicity was used to assess the lethal effects of Clove oil (CO) and clove oil nanoparticles (CO-NPs) on the laboratory DBM bioassay. Bioassay by leaf dip method, where fresh cabbage leaves were immersed in a solution of clove oil (CO) and clove oil nanoparticles (CO-NPs). Clove oil was previously dissolved in N-Hexane at concentrations ranging from 0.075 to 1.2%, while CO-NPs was dissolved in distilled water at concentrations ranging from 0.9 to 14.4%. It was then air-dried for 30 min before being placed in a 9 cm in diameter Petri dish that already contained 20 larvae of third instar DBM. Each treatment was carried out three times. At 24 and 48 h following application, mortality was observed.

### STATISTICAL ANALYSIS

To calculate the proportion of corrected mortality, the Abbott's formula was utilized (Abbott 1925). The means were determined using the Kruskal-Wallis (KW) nonparametric analysis of variance approach in SPSS 16. Probit analysis is used to calculate the $LC_{50}$ and $LC_{90}$ values of mortality data at 24 and 48 h using the statistical program SPSS 23.0. The experimental data of nanoparticle size, PDI, $LC_{50}$, and $LC_{90}$ were analyzed using a paired t-test at the 0.05 level.

## RESULTS AND DISCUSSION

### SIZE AND POLYDISPERSITY INDEX (PDI) OF CO-NPS

The 10% CO-NPs had an average size of 179.98 nm and a PDI of 0.246, according to the DLS tool. Table 1 summarizes the findings. According to these findings, the CO-NPs has a low PDI (below 0.3) and is nano-sized. Nanoparticles are microscopic particles ranging in size from a few nanometers to 1000 nanometers (nm) in diameter (Sinha et al. 2013). An appropriate chemical concentration is required to achieve the smallest particle size. The clove oil to PEG ratio with the lowest PDI, smallest size, and highest clove oil loading efficiency was 10% (Ikawati et al. 2021a). Molecular mobility, thermodynamic properties, environmental stress, preparation techniques, and conditions all influence the physical/chemical stability of the amorphous state (Baghel et al. 2016). The average of particle size and PDI in this study has a value that is almost the same as the previous study, which is the average size around 175 nm and the PDI below 0.3 (Ikawati et al. 2021a).

Figure 1 depicts the shape of the nanoparticles' size distribution. For three repetitions, the nanoparticles solution had a unimodal size distribution (Figure 1(A)), but for two replicates, it was bimodal (Figure 1(B)). A unimodal probability distribution, commonly referred to as a single-peak probability distribution, has only one peak, whereas a bimodal probability distribution has two peaks. These results are similar to those of Ikawati et al. (2021b), where the CO-NPs showed a bimodal and unimodal size distribution. Although the bimodal distribution comprises two peaks, the strength of the second peak is minimal (less than 10%), and the peak is an edge peak distribution. This implies that the second peak is an outlier, as it is an extra peak near the distribution's boundary that is out of place (Ikawati et al. 2020). So that the size distribution tends to be unimodal the same as the results of research Yang et al (2009), where size distributions of PEG coating nanoparticles loaded with garlic essential oil showed a unimodal size distribution.

Inherent and process-related physical features are important (Labuschagne 2018). Solid dispersion techniques have spurred interest in improving the dissolving rate of highly lipophilic compounds and thereby boosting their bioavailability by reducing particle size, improving wettability, and creating amorphous particles (Das et al. 2012). The bioavailability of weakly water-soluble drugs was dramatically increased using a solid dispersion formulation that included a water-soluble polymer and a surface-active agent (Dannenfelser et al. 2004). Inert, hydrophilic, pharmaceutical carrier matrices are a potential benefit of polymers (stabilization and solubilization) in solid dispersion (Baghel et al. 2016).

The fusion method is often used to describe the melt approach, which is accurate only when the starting components are crystalline (Das et al. 2012). Polymers can be amorphous (polyacrylic acid), semicrystalline (poly L-lactic acid), or crystalline (polyethylene glycol) (Baghel et al. 2016). To address the requirements of these novel applications, synthetic water-soluble polymers have been created with features never before realized in natural polymers. It is common practice to use reactive functional groupings to handle specific difficulties. Because of their architectural flexibility, water-soluble polymers play an essential role in nanotechnology and smart materials (Halake et al. 2014). As a result, PEG-based solid dispersion is one of the most promising methods for enhancing the bioavailability of chemicals that are difficult to dissolve in water, such as botanical pesticides. The PEG is widely used in many fields because of its water-soluble properties, biological compatibility, and nonimmunogenicity (Kacar 2018). As a result of recent breakthroughs in nanotechnology of 2D nanomaterials like graphene, PEG and its copolymers have been produced and used as dispersion agents (Halake et al. 2014).

TABLE 1. Clove oil nanoparticles' (CO-NPs) average size and polydispersity index (PDI)

| Sample | Z-Average (nm) | PDI |
|---|---|---|
| 1 | 187.5 | 0.296 |
| 2 | 177.8 | 0.223 |
| 3 | 184.5 | 0.271 |
| 4 | 176.3 | 0.233 |
| 5 | 173.8 | 0.209 |
| Average | 179.98 | 0.246 |

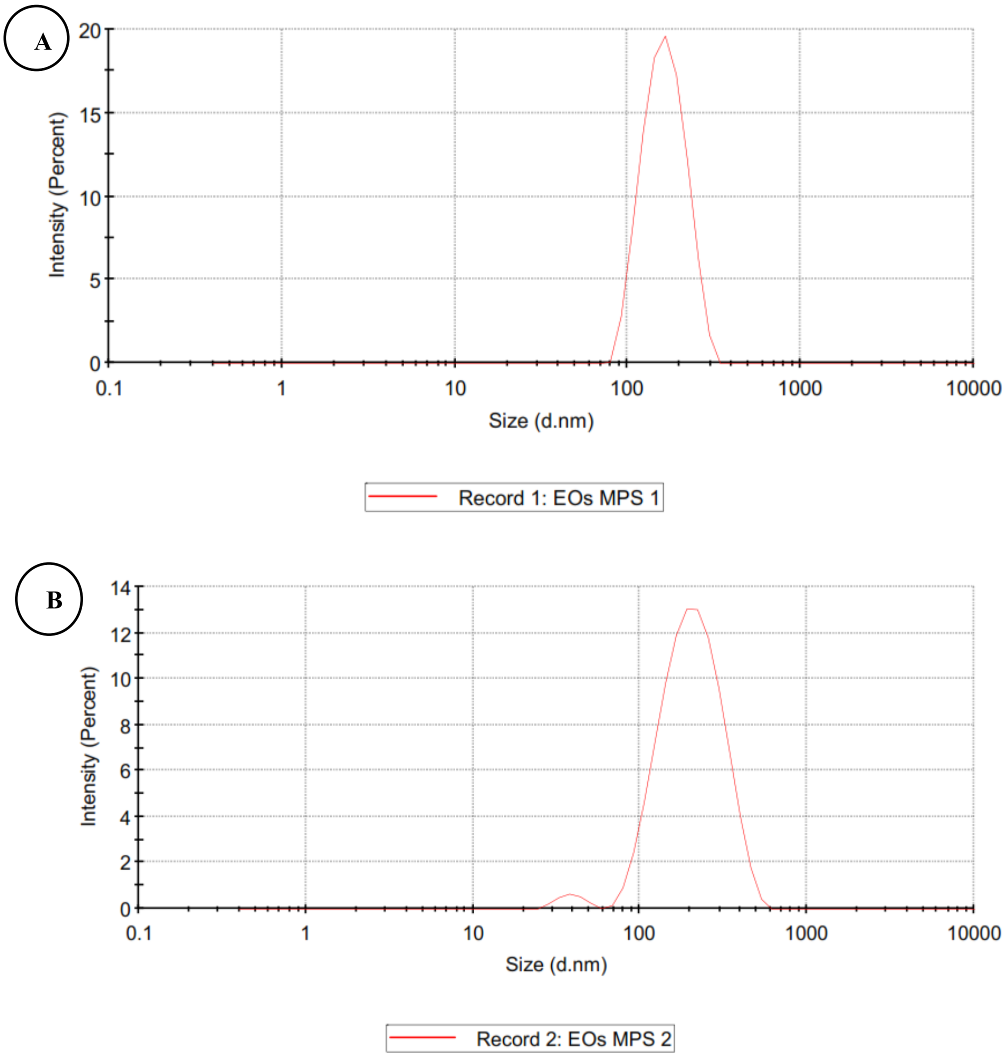


FIGURE 1. Dynamic light scattering was used to assess the distribution of CO-NPs size. A. Size distribution that is unimodal. B. Size distribution that is bimodal

### CHEMICAL COMPOSITION OF CLOVE OIL BEFORE/ AFTER NANOFORMULATION

Clove oil is a fragrant essential oil derived from the clove plant's flowers, stems, and leaves. The leaves clove oil utilized in this investigation was produced by a commercially available water distillation technique. Steam distillation can be used to obtain essential clove oil (Durán-Lara et al. 2020). In the pre-nanoformulation of clove oil, there are two primary components: eugenol and trans-caryophyllene (Table 2 & Figure 2). These findings corroborate prior data on clove oil's main constituents. The eugenol and caryophyllene content of clove essential oils was discovered to be high (Hossain et al. 2012). Eugenol is a member of the alkylbenzene family with an alkyl link ($C_{10}H_{12}O_2$) or 2-methoxy4- (2-propenyl) phenol. Its quantity in oil is mostly determined by the quality of raw materials and oil refining procedures (Widayat et al. 2014). In this study, eugenol content from leaves of clove oil was 83,87%. For trans-caryophyllene was 11,26. The primary constituents in clove oil leaves were eugenol (85.4%) and trans-caryophyllene (9,2%) (Ikawati et al. 2020).

Analysis of the results from GC-MS for the main compound eugenol, this compound decreased slightly after nanoformulation, which can occur due to oxidation or slight damage. Meanwhile, after the nanoformulation process, the content of the trans-caryophyllene compound increased slightly. However, there was no substantial chemical alteration between pre- and post-nanoformulation clove essential oil, according to the findings. These findings support prior claims that PEG 6000 might stabilize clove oil in the polymer, allowing it to minimize volatility considerably (Ikawati et al. 2021b). Due to their synergy, a combination of wall materials provides greater protection (Labuschagne 2018).

### TOXICITY OF CO AND CO-NPS AGAINST DIAMONDBACK MOTH (DBM)

Based on the Kruskal-Wallis nonparametric test, there were differences in the influence of the level of CO and CO-NPs concentration on the percentage of mortality after 24 and 48 h of treatment (P-value $<0.05$). Essential oils found in plants have been shown to have insect repellant, attractant, or insecticidal properties (Gross et al. 2014). Clove oil has the most documented bioactivity against insects and other pests of any essential oil (eugenol) (Durán-Lara et al. 2020). Residual toxicity of CO and CO-NPs increased gradually with increasing concentration. The CO treatment at the highest concentration resulted in fairly high toxicity, with 51% and 74% after 24 and 48 h of treatment (Figure 3). For the CO-NPs treatment at the highest concentration also resulted in fairly high toxicity of 78% and 85% after 24 and 48 h of treatment (Figure 3). This indicates the release of clove oil even though it has been nano formulated.

In this study we determined dose-mortality relationships of DBM to CO and CO-NPs. we determine not only $LC_{50}$ value but also $LC_{90}$. The $LC_{50}$ values of CO-NPs and CO for DBM larvae acquired from toxicity data showed that the nanoformulation had an effect on the $LC_{50}$ value, which describes its toxicity value, after 24 h of exposure (Table 3). The median lethal concentration ($LC_{50}$), also known as the median tolerance limit, is the level at which half of the test population dies (Balakrishnan et al. 2014). Because the $LC_{50}$ value of CO-NPs is lower than that of CO, it may be deduced that CO-NPs have a higher toxicity than CO. Meanwhile, the nanoformulation had no influence on the $LC_{50}$ value after 48 hr of exposure. There was no significant difference between the CO-NPs and CO treatments in terms of $LC_{90}$ after 24 and 48 h of exposure, which means there is no increase in toxicity in the nanoformulation.

TABLE 2. Chemical composition of clove oil before and after nanoformulation, as well as the percentage of primary component content

| Compound | Pre-formulation | | Post formulation | |
|---|---|---|---|---|
| | Retention time | Percentage | Retention time | Percentage |
| Eugenol | 20.692 | 83.87 | 22.501 | 75.23 |
| trans-Caryophyllene | 22.501 | 11.26 | 22.388 | 14.04 |

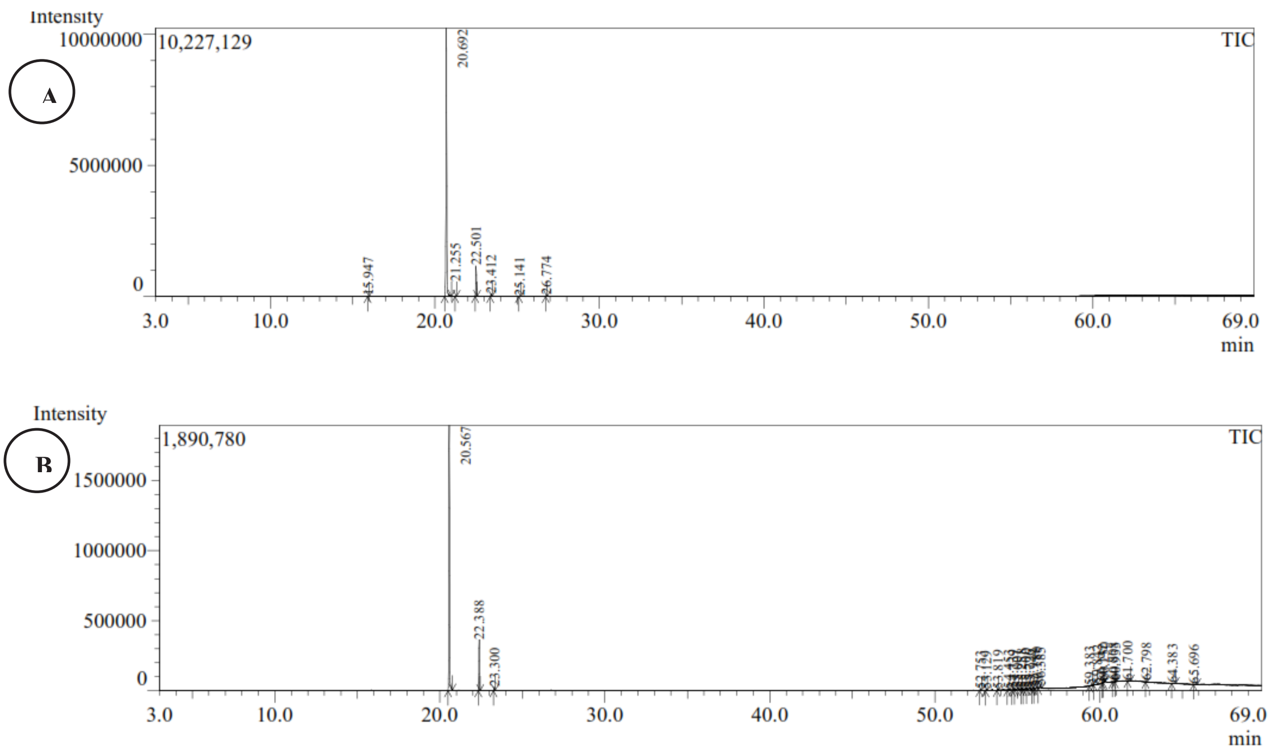


FIGURE 2. Clove oil (CO) and clove oil nanoparticles chromatogram (CO-NPs). CO chromatogram (A). CO-NPs chromatogram (B). Retention time 20.692 min (A)/20.507 min (B) for eugenol; Retention time 22.501 min (A)/22.388 min (B) for trans-caryophyllene

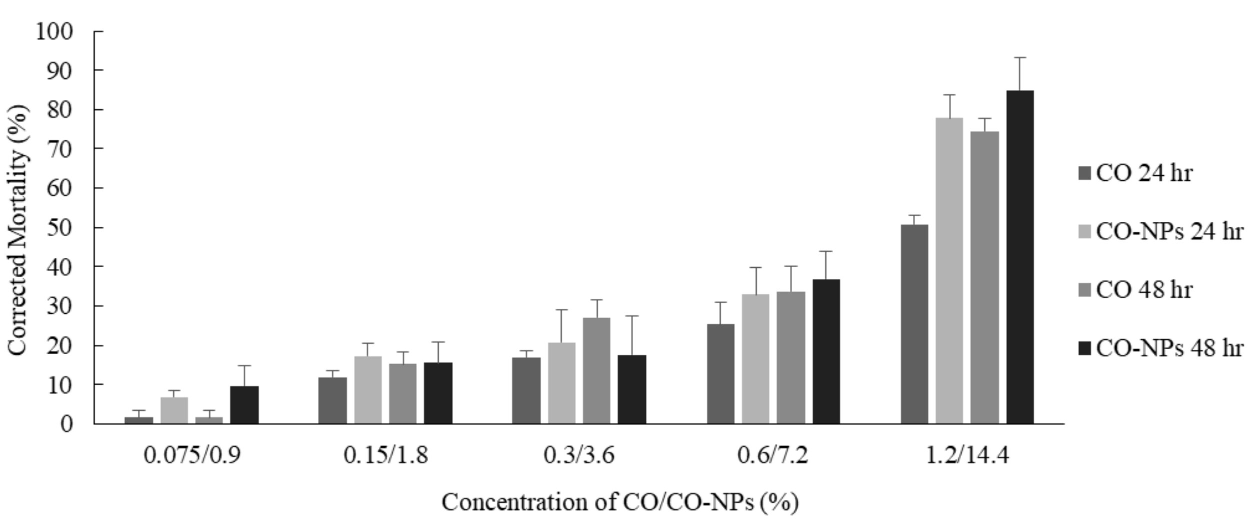


FIGURE 3. The effect of CO and CO-NPs on the proportion of DBM larvae mortality after 24 and 48 h of treatment

TABLE 3. CO-NPs and CO $LC_{50}$ and $LC_{90}$ values in DBM larvae

| Treatment after | $LC_{50}$ (%) | | $LC_{90}$ (%) | | Regression Equation (Y = a + bX) | |
|---|---|---|---|---|---|---|
| | CO-NPs | CO | CO-NPs | CO | CO-NPs | CO |
| 24 h | 10.308 a | 1.298 b | 20.901 a | 11.029 a | Y= -1.51 + 1.65X | Y= -015 + 1.6X |
| 48 h | 9.451 a | 0.658 a | 17.01 a | 3.376 a | Y= 1.24 + 3.05X | Y= 0.37 + 1.05X |

* On the 5% t-test, values preceded by various lowercase letters show statistically significant differences between the means in each column

The CO-NPs concentration response from regression equation had slopes (b) of 1.65 and 3.05 for 24 and 48 h of exposure. The CO slopes was also quite high, with values of 1.6 and 1.05 for 24 and 48 h of treatment, respectively. A high slope value illustrates that little changes in essential oil concentrations result in large variation changes in mortality (Pinheiro et al. 2013). Because of their high surface area, nanoparticles have different characteristics than bulk materials (Singh & Dutta 2018). The toxicity of clove oil nanoparticles to DBM increased after 24 h of exposure, as seen by the $LC_{50}$ value. The value of CO-NPs $LC_{50}$ is 10.308 % which is equal to 0,859% of CO, while the $LC_{50}$ value of clove oil is 1.298%. The same nanoparticle process was used, but with various essential oils, particularly garlic oil (Yang et al. 2009), geranium and bergamot oils (González et al. 2014) which showed increased in contact toxicity to *Tribolium castaneum*. This differs from a prior study in which CO-NPs was used to treat storage pests *C. ferrugineus* and *T. castaneum* and demonstrated a reduction in toxicity. The increase in toxicity is thought to be due to the main route of CO-NPs being as a stomach poison so that its toxicity is maximal for DBM compared to storage pests where the most effective route is fumigant poison which is lost due to nanoformulation. In some study showed that the toxicity of nano insecticides higher than conventional formulations (Balaji et al. 2015; Saini et al. 2014), although these findings have not been confirmed across a wide range of hosts or pest organisms (Kah et al. 2018).

The clove nanoparticles may be easily dissolved in distilled water for the leaf dip procedure in the toxicity test. So, based on the results of this study and previous study (Ikawati et al. 2021a), clove oil nanoparticles can dissolve well in water. PEG is thought to be in an amorphous condition, similar to what was found in the prior investigation (Ikawati et al. 2021a). The solubility of the amorphous form is expected to be higher than that of the crystalline form (Kanaujia et al. 2015). The PEG is referred to as polyethylene oxide (PEO) and depending on its molecular weight (Halake et al. 2014). Related to the elastic properties of PEG in dry and wet circumstances, it is obvious that PEG becomes less compressible and more elastic when water is added (Kacar 2018). Hydrophilic functional groups, such as ether, alcohol, amide, and pyrrolidone, are found in water-soluble synthetic polymers and are often friendly and nontoxic (Halake et al. 2014). PEG's strong polarity increases hydrophilicity and, as a result, water solubility, distinguishing it from other structural polymers (D'souza & Shegokar 2016). It also has a high solubility in a variety of organic solvents, a low melting point (below 65 °C), is non-toxic, can dissolve specific compounds, and can raise compound humidity (Koh et al. 2013). Polyethylene glycol is considered to be one of the most effective universal transporters for insoluble compounds (Wünsch et al. 2021).

## CONCLUSIONS

All of the studies provided here suggest that CO-NPs could be useful in the management of DBM, as the nanoformulation can boost clove oil's toxicity to DBM. More research is needed to determine its long-term toxicity to DBM and also other bioactivity.


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