Chlorpyrifos organophosphate and essential oils activities against *Callosobruchus maculatus* (F.) warehouse pests

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**Abstract.** *Callosobruchus maculatus* (F.) is a warehouse pest causing damage to foodstuffs, especially beans. One of techniques that usually used for preserving foodstuffs from insect’s attack in Indonesia is by using chemical pesticides and fumigants. The continuous use of chemicals can disrupt human health and pollute the environment. Pest control using essential oils is considered safer than chemicals and environmentally friendly. The essential oils that used in this study were *A. nardus*, *E. globulus*, and *M. piperita*. Nanoemulsion essential oils were made to reduce evaporation. The aim of this study is to find out the insecticidal activity of chlorpyrifos and three kinds of essential oils against *Callosobruchus maculatus* (F.). Research methods of this study consisted of sample preparation, fabrication of essential oils, mortality test and repellency test. The results showed that chlorpyrifos pesticides still have higher percentage of against insecticides compared to essential oils, but their use was not environmentally friendly.

1. **Introduction**

An important commodity in Indonesia is legumes or beans. The need for beans increases by an average of 2.8% a year, while the average production is still 1.0% a year [1]. This fact showed the deficiency of domestic beans production during the period ahead and can influence on the increasing of beans import. Post-harvest storage is the most important and influential stage of agricultural yield. At this stage, there is an interaction between environmental conditions and organisms, including warehouse pests, that can change the quality and quantity of foodstuffs. Warehouse pest can cause damage of foodstuffs and have an important role in human health [2]. One kind of warehouse pest that damages foodstuffs especially beans is *Callosobruchusmaculatus* (F.) [3]. This pest attacks the quality of legume or beans.

Pesticide is one of the main alternatives that used by farmers against warehouse pests because its effectiveness than biological or physical preventions. The variety of chemical pesticides usually used were made from organophosphate, one of them is chlorpyrifos. The use of chlorpyrifos insecticide (O, O-diethyl O-3,5,6-trichloro-2-pyridylphosphorothioate) has increased in agriculture, pest control and industry. The high level of toxicity of chlorpyrifos compounds is used as a reference to kill pests and insects with high intensity. Some studies show the importance of these chemicals to control warehouse pests [4]. Continuous use of chemicals can disrupt human health and pollute the environment. Fumigants are easily absorbed by plants that are dangerous when consumed by humans. Therefore, testing of
insecticidal activity and repellency of vegetable pesticides is needed to replace the chemical pesticides. There are various types for controlling warehouse pest using vegetable pesticides. Essential oils derived from plants are very promising for controlling *Callosobruchus maculatus* in storage units [5]. Some examples of essential oils that have been investigated as vegetable insecticides include galangal (*Alpinia galanga*), lemon grass fragrant (*Cymbopogon nardus*), and cloves (*Syzygium aromaticum*) which are effective for controlling pepper flower sucking *Diconocorishewetti* [6]. Essential oils that used in this study were *Andropogon nardus*, *Eucalyptus globulus*, and *Mentha piperita*. Lemongrass have potential to be biodegradable and environmentally friendly pesticides [7]. In addition, *Eucalyptus globulus* and *Mentha piperita* can be used as insecticides and pest repellent [8]. Essential oils compound is volatile, so that the technology that can reduce the evaporation is needed. Essential oil nanoparticle technology can use to reduce evaporation and increase the effectiveness of warehouse pest control. The advantages of nanoparticle technology are non-toxic, stable during use, wide surface area and can be used as a matrix for various types of drugs and plant extracts [9]. The aims of this study is to determine the insecticidal activity of chlorpyrifos and three types of essential oils against the *Callosobruchus maculatus* imago.

2. Materials and methods
This research was conducted at Laboratory of Biochemistry and Termite Laboratory, Biology Department, Universitas Negeri Semarang at October-December 2018. The sample in this study was 780-imago stage 3 days of *Callosobruchus maculatus* (F.). The equipment used in this study is erlenmeyer, cup glass, test tube, petridish 9 cm in diameter, micropipette, pipette, 500 ml reagent glass bottle, stopwatch, magnetic structure and glass stirrer. The materials that used in this study are *Atropogonnardus*, *Eucaliyptusglobulus*, and *Menthapiperita* essential oils, chlorpyrifos, green beans, chitosan, tween 80 0.1%, acetone, aquades, Whatman paper No. 1, glacial acetic acid and sodium tripolyphosphate (STPP).

2.1. Preparation of *Callosobruchus maculatus* (F.)
The colonies of *Callosobruchus maculatus* (F.) were obtained from filtered media on bean medium *Vigna angularis* in glass sheets which were covered with tile. Beans infestation is rearing at 27°C, 70% relative humidity and separated by sex for the next stage.

2.2. Essential oil nanoparticles fabrication
The nanoparticle preparation was carried out by dissolving 3 grams of chitosan in 100 mL of 1% liquid acidic acid and homogenizing at 2000 rpm for 2 hours. The homogeneous mixture was added with 20 μL tween 80 0.1% and homogenized again at 2000 rpm for 30 minutes. Next, 200 mL of STPP 0.1% and 2 mL, 4 mL, 6 mL, and 8 mL were added to each of *Atropogonnardus Eucaliyptusglobulus*, and *Menthapiperita* essential oil. Mixing this mixture and homogenized at 2000 rpm for 30 minutes.

2.3. Preparation of chlorpyrifos and essential oils
Chlorpyrifos pesticides in this study are in the form of powder that dissolved in distilled water. Chlorpyrifos is diluted to four levels of concentration namely 2, 4, 6, 8%. Dilution of essential oils from each type of plant was dissolved with nanoparticle chitosan with the same level of concentration with chlorpyrifos pesticides.

2.4. Mortality test
The mortality of the *Callosobruchusmaculatus* (F.) imago was tested by the fumigation method using filter paper with chlorpyrifos pesticides and essential oils. The essential oils of chlorpyrifos pesticides used for fumigation were at four levels of concentration, namely 2, 4, 6, 8% plus controls. Each treatment was repeated three times. Each concentration of essential oils and chlorpyrifos was pipetted 0.5 ml and dripped equally on filter paper which had been glued to the surface inside the lid of petri dish. Control filter paper was dripped with 0.5 ml of acetone. Dripped of the essential oils solution is carried out
equally from the outside inward. The filter paper is left for 1 minute after the treatment to evaporate the acetone solvent. After drying, 10 images are put into the petri dish. Then the petri dish is tightly closed, the gap between the lid and the base of the petri dish is sealed with plastic wrap to prevent oil vapor leakage. Observation of insect mortality is carried out after 24 hours and 72 hours of fumigation [10]. The observation parameter used in this study was the percentage of mortality of the *Callosobruchus maculatus* (F) imago (%).

3. Results and discussion
The percentage of mortality of *Callosobruchus maculatus* (F.) which is fumigated using essential oil within 24 and 72 hours. In the 24 hours treatment, the highest mortality of essential oils treatment was found in the *Eucalyptus globulosa* at 65%. Followed by *Mentha piperita* 56% and *Anthropogon nardus* 53%. In the 72 hours period, the percentage of deaths has increased, showed in the figure 1. *Mentha piperita* essential oil has stronger toxicity than its contact toxicity [11], and by using nanoemulsion techniques can increase contact activity through slow release of active ingredients over time [12].

The percentage of insect mortality within 24 hours that was fumigated with chlorpyrific pesticides at concentrations of 2, 4, 6, and 8% were 80, 85, 100, and 95%. At 72 hours, the percentage of test insect deaths at concentrations of 2, 4, 6, and 8% is 100%, that showed in Figure 1. Contact toxicity can disturb the nervous system’s work in pests that cause cell paralysis muscle, lead to the pests to stop eating and die [13]. It can be said that chlorpyrifos pesticides cause faster death in *Callosobruchus maculatus* (F.) than essential oils. However, the use of essential oils as vegetable pesticides is considered safer. From the results of the Independent Sample T Test, the difference between both of them is not significant.

In the study essential oils used in the form of nanoemulsion. Nanoemulsion with chitosan method can improve essential oil’s performance. Nanoemulsions of various types have better physical stability and longer resistance [14]. The stability and bioavailability of peppermint oil can be used as fumigants and antidotes in *T. confusum* [15], rejecting contact activities of *Sitophilus oryzae* L. [16], and oviposition of latency to *Callosobruchus maculatus* (F.) [17]. In fact, nanoemulsion can increase the stability of essential oils *M. longifolia*.

![Figure 1](image)

Figure 1. Mortality percentage of *Callosobruchus maculatus* (F.) with essential oils treatment for a) 24 hours treatment, b) 72 hours treatment.

4. Conclusion
*Callosobruchus maculatus* (F.) mortality with chlorpyrifos and *Eucalyptus globulus* pesticide 8% treatment after 72 hours is 100%. Both of them have a high insecticidal property. *Anthropogon nardus* and *Mentha perita* pesticide 8% gave 75% mortality after 72 hours treatment. It can be conclude that the three essential oils are potential to against *Callosobruchus maculatus* (F.).

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