The Effectiveness of Suspension Beta Asarone Mixed with Sillica Nanoparticles in the Mortality of *Crocidolomia pavonana*

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Abstract. The prospect of using botanical insecticides to control the insect pests is very promising. However, there are some factors limiting their efficacy. Short release rate at the point contact, the inherent volatility and vulnerability to oxidation and ultra-violet light are causing phytochemical changes during the application. The use of nanoparticles is a novel technology with their potency to maximize the efficacy of phytochemicals. One of the promising plants contained insecticidal compound is *Acorus calamus.* The main compound of *A. calamus,* α and β-asarone with the latest is predominantly. The improvement of β-asarone on silica nanoparticle was evaluated against the cabbage heart worm (*Crocidolomia pavonana).* The bioactivity of the nanoformulation was identified by the larvae mortality using immersion methods. Several findings have been achieved. In term of findings, it showed that β-asarone formulated in silica nanoparticles can increase their efficacy. The formulation reduced the insect development and reduced the number of survived insects. The synergistic effect found on the formulation. This can be determined from the increasing of the number of death larvae and pupae.

Keywords: Silica nanoparticles · β-asarone · Reduced the mortality · Crocidolomia pavonana

1 Introduction

Insecticide is a compound used to control insects, especially those that act as pests and diseases in plants. The method of control is usually using synthetic insecticides. However, the application that are not in accordance with the rules will have a negative impact on the environment [1]. The negative impact caused is resistance to target insects. Another negative effect is the presence of residues that are harmful to other organisms so that they are not environmentally friendly [2].

An alternative method used to reduce the impact of synthetic insecticide application is using botanical insecticides [3]. One of the plants that produces insecticidal compounds is the rhizome of the dringo plant (*Acorus calamus*). The rhizome of *A. calamus* contains

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compounds of asarone, cholin, flavones, acoradin, galangin, acolamone and isocolamone which are volatile and rapidly degraded in nature [4].

The volatile and rapidly degraded or unstable nature of botanical insecticides can be caused by concentration and pH of the formulation [5]. According to Schummuterer [6] botanical insecticides which is containing the active compound azadirachtin showed varying degrees of sensitivity to environmental factors such as high temperature, exposure to ultraviolet light, pH and rainfall applied to plant tissues. In the United States, botanical insecticides containing azadirachtin have been equipped with UV-inhibitors, this aims to maintain the stability and sensitivity of botanical insecticides [7].

The weakness of the natural botanical insecticidal compounds is a challenge in developing β-asarone formulations in silica nanoparticles with 0.1% concentration and 30 days absorption. One of the developments is by combining botanical insecticides with other compounds or elements that are more stable in nature. One element that has good absorption is silica. Silica nanoparticles have properties that are easy to bind and are insecticidal. Silica in nanoparticles can act as an insecticide because it can damage the cuticle layer of *Sitophilus oryzae* [8]. The existence of this combination can cause botanical insecticides more efficient and not easily decomposed by abiotic factors. The use of silica nanoparticles can induce compounds from botanical insecticides so that they are not easily oxidized by abiotic factors such as UV light [9].

Currently, a study was conducted on botanical insecticides in β-asaron compounds with silica nanoparticles at different concentration units and absorption times [10] (Unpublished Data). One of them is the formulation of β -asarone in silica nanoparticles with 0.1% concentration and 30 days absorption. Based on this study, it can be assumed that it can affect the mortality of *Crocidolomia pavonana.*

C. pavonana is an insect pest that attacks cabbage plants and causes plant damage starting from age 10 week after planting aged 10 weeks after planting [11]. *C. pavonana* larvae usually attack seedling and cause that it can cause death in cabbage plants [12]. Therefore, it is necessary to carry out a biological assay to determine the effect of the β -asaron formulation in silica nanoparticles with 0.1% concentration and 30-day absorption on the mortality of *C. pavonana.*

2 Materials and Methods

2.1 Characterization of β Asarone in Formulated Silica Nanoparticles

Nanoparticles used for this project is silica. Comparing to bentonite as nanoparticles [10] silica or silicon dioxide nanoparticles have more broad range on biological application and mostly used as an entomotoxic agents, such as *Sitophilus oryzae* [8].

Nanoformulation was prepared by absorbing β asarone onto the silica nanoparticles by using immersion method. 100 mg of silica nanoparticles dispersed in a volumetric flask containing 6 ml chloroform. An appropriate amount of asarone was separately mixed to obtain 4 different concentrations (0.025%, 0.05%, 0.1%, 0.2%). The suspension then sonicated for 10 min then agitated for 24 h at 20 rpm. Chloroform was evaporated at room temperature then vortexed for 20s. The resulted formulation placed in airtight pouches and stored at 25ºC in desiccators. The β-asarone adsorbed silica nanoparticles will be used in chemical analysis and bioassays.

2.2 Investigate the Efficacy of Insecticidal Formulated by Toxicity Bioassay

The method used in this research is the immersion method. Cabbage leaves were cut into small pieces with a diameter of 3 cm using a perforator. The leaves were immersed in the treatment solution, namely control (0% distilled water), 0.1% silica nanoparticles and the formulation of -asarone in 0.1% silica nanoparticles and 30 days absorption. The cabbage leaves are dipped for 1 min, then the leaves are air-dried for 5 min. The cabbage leaves were then put in a plastic tube (Ø = 5 cm and t = 7.8 cm) in which filter paper was added. In each tube, 10 larvae of *C. pavonana* instar 2 were put using a brush. The larvae had previously been fasted or not fed for 3 h so that when applied, the larvae could respond quickly to the treatment. Furthermore, *C. pavonana* was recorded and observed 24 h after application until all the imagoes died, the mortality observations were carried out at the 2, 3, 4 instars, pupa and imagoes. Each treatment was repeated 15 times.

Data obtained were analyzed using the analysis of variance test for the difference in the values of the control and treatment groups using Two Way Anova and followed by Duncan with a 95% confidence level ($\alpha = 0.05$) using SPSS 16.0 for Windows.

3 Results and Discussion

3.1 Effect of β-Asarone Formulation in Silica Nanoparticles on Larval Mortality of *C. pavonana*

The results showed that there were differences in the effect of several formulations on the mortality of *C. pavonana* larvae ($F = 5.442$; $P = 0.000$) (Table 1).

All the formulations showed that the larval mortality had increased with the length of time of application. The mortality of test larvae at 24 h after application showed the same value in the β-asarone formulation in silica nanoparticles and silica nanoparticles, which was 5%. The mortality rate was higher than the control. Meanwhile, the mortality larvae after 72 h showed that the β -asarone formulation in silica nanoparticles was higher (11%) as compared to silica nanoparticles (9%). The mortality of test larvae was higher at 216 h after application. It can be observed in all formulations that β -asarone in silica nanoparticles caused the death of the larvae (47%) while the silica nanoparticle

Table 1. Average mortality of *C. pavonana* larvae at 24, 72 and 216 h

Treatments	Mortality (%) ± SD		
	24 h (2^{nd} instar)	72 h (3^{rd} Instar)	216 h (4^{th} Instar)
β-asaron in silica nanoparticle formulation with concentration 0.1%	$5 \pm 7.07^{a(p)}$	$11 \pm 9.94^{a(p)}$	$47 \pm 12.52^{a(q)}$
Silica nanopartikel with concentration 0.1%	$5 \pm 7.07^{a(p)}$	$9 \pm 11^{a(p)}$	$40 \pm 9.43^{a(q)}$
Control 0% (aquades only)	$0 \pm 0^{b(p)}$	$0 \pm 0^{b(p)}$	$8 \pm 9.19^{b(q)}$

Notes: Different superscript letters show significantly different values in the Two-Way Anova test and Duncan's follow-up test with a 95% confidence level (n = 15).

formulations caused death (40%). The mortality of the larvae in the control was only observed after 216 h of application, namely 8% (Table 1).

Further test analysis (Duncan $\alpha \leq 5\%$) showed that all formulations were significantly different from the control. However, between treatments showed no significant difference. Based on the time of application, all treatments and controls at 24 h and 72 h after application showed no significant difference. However, at 216 h after application, the observation time between formulations showed a significant difference compared to the observation time 24 and 72 h after application.

The death larvae of *C. pavonana* showed that the body shriveled and blackish brown in color. Meanwhile, the death larvae in the control did not shrivel and having a yellow-green color (Fig. 1). The color change in the test larvae that died due to the influence of the treatment was thought to be caused by the melanization process. The melanization process is a form of insect defense mechanism against foreign compounds that enter the body [13]. The sixth instar larvae of *Spodoptera exigua* treated with 10 mM diisopropyl fluorophosphate or trypsin inhibitor soy orally, could inhibit the activity of the phenoloxidase enzyme [14]. The role of the phenoloxidase enzyme in hemolymph as a humoral immune response in the insect's body if there are foreign compounds that enter the insect's body [15]. The melanization process is influenced by the phenoloxidase enzyme present in hemolymph. The initial stage of the protein serine protease cascade as a precursor to activate the prophenoloxidase enzyme, then the enzyme is converted into an active form into the phenoloxidase enzyme. The phenloxidase enzyme oxidizes phenol and tyrosine into quinones contained in the cuticle of insects, resulting in a color change process or a melanization process [16].

The formulation of β-asaron and silica nanoparticles is thought to have a synergistic effect. This can be seen from the value of the highest percentage of deaths. Several studies have stated that there is a synergism between nanoparticles and active compounds from plants. Lian et al. [17] reported that the use of polyethylene glycol nanoparticles with garlic essential oil against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) showed imago mortality (11%) higher, than the essential oil treatment alone and controls. According to Rouhani et al. [18] the application of a mixture of imidacloprid, Ag and Zn nanoparticles at a concentration of 1 L mL^{-1} and nanoparticles at 700 mg mL-1 showed the highest mortality rate in *Aphis nerii* (Hemiptera: Aphididae). In addition, according

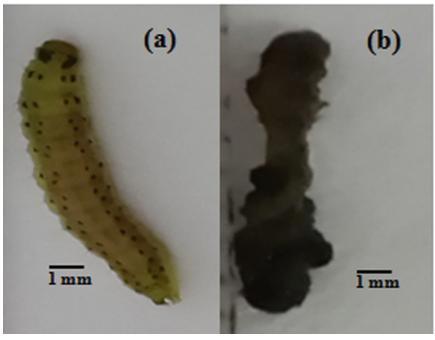

Fig. 1. Dead fourth instar *C. pavonana* larvae. (a) control; (b) treatment.

to Anbu et al. [19] there was a synergistic effect on the formulation of neem oil and 10% silver nanoparticles which caused the death of *Spodoptera litura* larvae up to 100% at eight days after application.

3.2 Effect of β-asarone Formulation in Silica Nanoparticles on Pupa and Imago Mortality of *C. pavonana*

The effect of β-asarone formulation in silica nanoparticles on pupa and imago mortality showed the difference effect of treatment and control on the mortality of *C. pavonana* pupae. The β-asarone in silica nanoparticles, 408 h after application, showed the highest mortality (64%) than silica nanoparticles alone (55%) and control treatment (12%). Interestingly, the number of malformed pupae and imago treated the β-asaron in silica nanoparticles was the highest (64%) among the control. The malformed pupae looked shriveled with the cuticle layer of the pupa was damaged and dark brown-black in color. While the pupa mortality in the control remained oval in shape. The malformed imago had rolled wings (Fig. 2).

Imago mortality at 672 h after application had the highest mortality percentage in the -asarone treatment in silica nanoparticles (88%) than in silica nanoparticles alone (80%) and control treatment (16%). During the development process, insects simultaneously produce juvenile hormones and ecdysone hormones. Both hormones are produced in the prothoracic glands. If the concentration of juvenile hormone is low, the ecdysone hormone is high [20]. β-asaron inhibit the work of juvenile hormones so that the process of skin turnover is disrupted and will cause the malformed pupae. Likewise, effect β-asaron on imago is also suspected to cause abnormality, because the insect's body system has been disturbed during the pre and pupa stage, causing *C. pavonana* did not able to emerge. In addition, the condition of the curled wings causes the imago unable to fly so it caused difficulty to find food.

The formulation of β-asarone in silica nanoparticles increased the efficacy against *C pavonana* larvae. This formulation also affects on malformed either in pupae or imago.

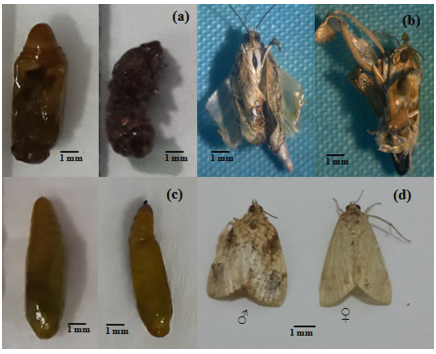

Fig. 2. Pupae and imago of *C. pavonana.* (a) abnormal pupae; (b) abnormal imago; (c) normal pupae; (d) normal imago.

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Authors' Contributions. P, BR, SF, and RJ designed the experiment. P performed the experiments. P and BR analysed the data P and RJ wrote the first draft of the manuscripts and P and RJ wrote with BR the final version. All authors read and approved the final manuscript.

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