Bioinsecticide activity of silver nanoparticles product of biosynthesis using entomopathogen against the beet caterpillar *Spodoptera exigua*

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ABSTRACT

Spodoptera exigua (Hubner) is a polyphagous insect pest that is detrimental to farmers. Nanotechnology exists as a renewable technology that can increase effectiveness due to the small size of particles it produces. This study aimed to determine the effectiveness of biosynthesis of silver nanoparticles from the fungi *Metarhizium*, *Trichoderma*, and The *Bacillus* bacteria which are biocontrol agents. The research started in February and ended in May 2020, at the Biological Research Laboratory, Faculty of Mathematics and Natural Sciences Education, University of Pendidikan Indonesia. The experiment was based on a completely randomized design method (CRD). Onion armyworm (*Spodoptera exigu*a), two isolates from the fungi *Metarhizium* and *Trichoderma* were obtained from the Indonesia Vegetable Research Institute (IVERRI). Each extract of *Metarhizium* and *Trichoderma* fungi and 50 mL of extract from *Bacillus* bacteria were added to 50 mL of Ag + 60 ppm solution in a 250 mL glass tube. The mixture of *Metarhizium* mushroom extract with 60 ppm Ag + solution or *Trichoderma* mushroom with Ag + 60 ppm solution was incubated at 40^0 C with a rotation speed of 200 rpm for five days under a dark condition. For the mixture of bacterial extracts with Ag + 60 ppm solution, incubation was at room temperature with a rotation speed of 150 rpm for three days. Analysis of the effect of NPP on the mortality of *S. exigua* was done using the treatments, *Metarhizum*, *Trichoderma*, *Bacillus*, NPP-*Metarhizium*, NPP-*Trichoderma*, NPP-*Bacillus*, NPP, and distilled water as controls. Third instar larvae of *S. exigua* were immersed for 30 seconds. After immersion, the larvae are placed in sterile Petri dishes that have been lined with sterile filter paper and provided with caterpillar feed. Observation of caterpillar mortality was recorded at 2, 4, 6, 9, 24, 48, 64, 72, 96 and 108 hours after treatment. There were three replicates the results showed that all the treatments caused death of the insect, both entomopathogens and entomopathogenic nanoparticles. We found the three treatments of silver nanoparticles highly lethal to *S. exigua*. Two treatments, NPP-*Metarhizium* and NPP-*Bacillus* even caused 100% mortality after 120 hrs treatment. The possible reason for the pathogenicity of these products is considered to be biosynthesis of nanoparticles and enzymatic substances

Keywords: Silver nanoparticles, *Spodoptera exigu*a, biosynthesis of silver nanoparticles, insecticide

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INTRODUCTION

Spodoptera exigua, commonly known in Indonesia as the beet armyworm, is a polyphagous insect pest most avoided by farmers. The presence of *S. exigua* can cause crop failure, leading to severe economic losses for the world (Talaee *et al.*, 2017). The damage caused *S. exigua* attack can be seen in transparent spots on the leaves due to the inner leaf tissue that has been eaten by the caterpillar, while the epidermal layer is left behind. In addition, the leaves that experience severe attacks from *S. exigua* will dry out and fall prematurely to decrease the quality and quantity of crop yields (Marsadi *et al*., 2017). Thus, its presence is detrimental, making farmers make various efforts to control it.

One of the efforts of farmers to control insect pests, especially *S. exigua*, is to apply synthetic chemical insecticides (Hastuti *et al.*, 2016). However, farmers often abuse synthetic chemical insecticides by increasing the dose or mixing one with another insecticide. Others, causing negative impacts on the environment and health of farmers (Marsadi *et al.*, 2017). Nanoparticle technology or nanotechnology produces a material that has a size of 1-100 nm (Maryani *et al.*, 2017). Silver nanoparticles (NPP) can be obtained using three synthesizing methods; physical, chemical, and biological. Biological NPP synthesis has many advantages, including being simple, fast, non-toxic, producing NPP with stable size and shape (Zhang *et al.*, 2016). Therefore, the existence of this nanoparticle technology gives hope in efforts to control insect pests.

Furthermore, many studies have discussed the effectiveness of entomopathogenic fungi and bacteria as pest control agents. However, not many studies have discussed the effect of biosynthetic NPP as a control method for *S. exigua*. It is therefore, necessary to determine the effect of NPP that has been synthesized using fungi and entomopathogenic bacteria against the onion armyworm (*S. exigua*). This study was aimed at determining the effect of NPP synthesized using entomopathogenic fungi and bacteria on the mortality or survival third instars larvae of *S. exigua*.

MATERIALS AND METHODS

This research was an experimental study based on a completely randomized design method (CRD). Before the research was carried out, the tools and materials were sterilized using an autoclave. The isolates of *Metarhizium* and *Trichoderma* fungi, *Bacillus* bacteria isolate and silver nanomaterials were obtained from the Indonesian Vegetable Research Institute (IVEGRI).

Preparation of microbial culture extracts

Pure Fungal of *Metarhizium*,*Trichoderma* and pure bacterial *Bacillus* obtain from IVEGRI

Synthesizing Silver Nanoparticles

Each extract of *Metarhizium* and *Trichoderma* fungi and 50 mL of extract from *Bacillus* bacteria was added with 50 mL of Ag + 60 ppm solution in a 250 mL glass tube (Plate 1A). First, the mixture of *Metharizium* mushroom extract with a 60 ppm Ag + solution or *Trichoderma* mushroom with Ag + 60 ppm solution was incubated at 40 ^{O}C with a rotation speed of 200 rpm for five days under dark conditions (Kamil *et al.*, 2017). The mixture of bacterial extracts with Ag + 60 ppm solution, incubated at room temperature with a rotation speed of 150 rpm for three days (Banu *et al.*, 2014). The colour change in the mixture was the first indicator that the nanoparticles have formed. After that, the mixture turned brown (Tyagi *et al.*, 2019).

Analysis of the effect of NPP on *S. exigua* mortality

Third instar larvae of *S. exigua* were immersed for 30 seconds in each treatment of *Metarhizuym*, *Trichoderma*, *Bacillus*, NPP-*Metarhizium*, NPP-*Trichoderma*, NPP-*Bacillus*, NPP, and distilled water as control (Cetin *et al.*, 2006). After immersion, the larvae are placed in sterile Petri dishes that have been lined with sterile filter paper and provided with caterpillar feed. Caterpillar mortality was recorded at 2, 4, 6, 9, 24, 48, 64, 72, 96 and 108 hrs after treatment. There were three replicates.

Data Analysis

The data analysis performed in this study was the Kruskal-Wallis testusing the IBM SPSS Statistics 24 application. The Kruskal-Wallis test is a non-parametric statistical test to determine whether the data that was obtained were statistically significant different between the treatments.

RESULTS

Biosynthesis of Silver Nanoparticles

The synthesis of silver nanoparticles (NPP) in this study used the biosynthetic method. Silver nanoparticle biosynthesis is an environmentally friendly synthesis method. NPP biosynthesis carried out in this study is a biosynthesis using extracellular microorganisms, namely by using extracts from microorganisms as reducers mixed with Ag + metal ions. NPP biosynthesis carried out in this study resulted in three types of NPP, including NPP-*Bacillus*, NPP-*Metarhizium*, and NPP-*Trichoderma*. The colour change that occured in the mixture of microorganisms with Ag + metal ions was the first indicator of the presence of silver nanoparticles. In this study, it was observed that there was a colour change from golden yellow to brown, indicating that silver nanoparticles had been formed. The characteristics of each NPP varied widely, depending on the material used. The NPP produced by *Trichoderma* fungus was generally 13-18 nm in size (Abdullaeva, 2017), NPP-*Metarhizium* 28-38 nm and NPP -Bacillus bacteria had a larger size when compared to the NPP produced by the fungi. The size of the NPP produced by *Bacillus* bacteria was ± 85nm (Sayed *et al.,* 2017).

Table 1. Bio Nanoparticles application to *Spodoptera exigua*

Treatment	Hours						Percentage
	24	48	72	84	96	108	
NPP-*Bacillus*	0	2	2	2	2	1	100
NPP-*Metarhizium*	0	2	2	2	2	2	100
NPP-*Trichoderma*	1	2	2	2	1	2	100
Bacillus	1	1	1	1	2	2	80
Metarhizium	1	1	2	2	1	1	80
Trichoderma	0	0	1	2	2	2	70
NPP	0	0	1	1	1	1	40
control	0	0	0	0	0	0	0

NPP on the mortality of *S. exigua*

Analysis of the effect of NPP on the mortality of third instar larvae of *S. exigua* is shown in Table 1. The table shows the average number of caterpillar deaths every hour after treatment, as shown in the table. The table also contains the percentage of caterpillar deaths that have been given each treatment.

Based on Table 1, all NPPs affected the mortality of the third instar larvae of *S. exigua*. Silver nanoparticles can be said to affect the mortality of third instar larvae of *S. exigua* because of the very striking differences in the mortality of third instar larvae of *S. exigua*, given NPP with third instar larvae of *S. exigua* in the control treatment. Based on the percentage of larval mortality, the NPP that greatly influenced the mortality of third instar larvae of *S. exigua* were NPP-*Trichoderma* and NPP-*Metarhizium*. Based on Table 1, one hundred percent of *S. exigua* third instar larvae died due to exposure to NPP-*Trichoderma* and NPP-*Metarhizium*. It showed that the ones that most influence the mortality of third instar larvae of *S. exigua* are NPP-*Trichoderma* and NPP-*Metarhizium*

DISCUSSION

Silver nanoparticles (NPP) synthesized through biological methods have now become an alternative in treating plant insect pests. It is based on the fact that the NPP produced is a compound that does not harm a safe environment. It has been known that silver ions are toxic, and it has been agreed that the toxicity possessed by silver ions contributes to the biological activity of NPP. Several studies have reported the influence of size, capping, concentration, temperature, pH, ionic strength, and time of NPP breakdown (Foldbjerg *et al.*, 2015).

In some cases, the toxicity of the nanoparticles to insects is difficult to understand. The mode of action of NPP against insects is through activating insect-target alteration pathways and triggering the release of toxins in the insect's stomach (Siddhardha *et al.,* 2020). Several studies have also discussed that some nanoparticles can act as "trojan horses" that pass through cell walls and then produce toxic ions to damage parts of the cells (Benelli, 2018).

Jyothsna Yasur and Usha Rani Pathipati conducted a study on the susceptibility of Lepidoptera insects to NPP. In their report, NPP decreased the growth index of *Spodoptera litura* and *Achaea janata* L. larvae. The accumulation of silver occurs in the body of the larva. It was found that the most accumulation of silver was in the faeces of the larvae and then the cuticles, and a minor accumulation was in the intestines. The insecticidal properties of NPP can be seen from the larvae's morphological appearance, which causes changes in their physiology. It is suspected that NPP's toxicity mainly occurs due to oxidative stress (Yasur and Pathipati, 2015). The latest knowledge about nanoparticles is that there is a well-accepted theory, namely the toxicity possessed by nanoparticles, namely their ability to trigger oxidative stress in arthropod tissue (Benelli, 2018). The oxidative stress experienced by larvae results in reactive oxygen species (ROS) (Yasur and Pathipati, 2015).

The statistical analysis that has been carried out in this study is the Kruskal-Wallis statistical analysis. The analysis results indicated that there were statistically significant differences between the treatments, which is indicated by an F value of $P < 0.002$. Data will be said to be significantly different when the significance value is less than 0.05. The percentage mortality of caterpillar in the NPP-*Metarhizium*, NPP-*Trichoderma*, and NPP-*Bacillus* treatments was significantly higher when compared to the *Metarhizium Trichoderma* and *Bacillus* treatments. There was a tendency for NPP to perform better as an insecticide when compared to fungi and bacteria alone. Therefore, the performance of NPP as an insecticide needs to be reviewed. Silver nanoparticles produced from biosynthesis using fungi and bacteria must have different infection pathways with entomopathogenic fungi and bacteria themselves.

Insects have an excellent innate immune response. The innate immune response possessed by insects consists of several defence mechanisms, including defence in the epithelium, local and systemic immune reactions, also insects involve the formation of ROS, at the site of infection to kill pathogens. Excessive ROS in an insect's body will cause damage to various cellular macromolecules such as proteins, lipids, and DNA. The damage that occurs due to the presence of excessive ROS is known as oxidative stress. Therefore, insects must balance the production and degradation of ROS to prevent fatal oxidative stress (Zug and Hammerstein, 2015). NPP is an agent that can penetrate several infectious mechanisms of the larvae. First, NPP can penetrate the epithelial defence mechanism possessed by the larva. It is likely due to the size of the NPP, which is so tiny that it can penetrate properly and penetrate directly into the larva's body, reaching the target organs. NPP, with a tiny size, has a vast touch surface area (surface to volume ratio). In other words, NPP can fill the surface of the target cell in larvae during the initial stage of infection.

The infection mechanism of NPP against *S. exigua* larvae does not escape the defence mechanism in the larva itself. The defence mechanism in larvae occurs when a foreign substance, namely NPP, enters the larva's body. Physiological changes that occur in larvae and cause morphological damage are thought to be due to NPP accumulation in several parts of the larva's body. The accumulation of NPP in the larva's body allows stress, resulting in changes in the enzymatic level in the larva's intestinal tissue. It leads to the claim that NPP can trigger the formation of reactive oxygen species (ROS) in the larvae.

Treatment of Silver nanoparticles (NPP), especially Silver nanoparticles-*Bacillus*, silver nanoparticles-*Trichoderma* and silver nanoparticles-*Metarhizium* treatment, were treatments with a mean proportion of larval mortality in the amount of 90 - 100% in 5 days

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