Biosynthesis silver nanoparticle using *Bacillus thuringiensis* strain BT2 and its potential use against *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae)

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Abstract. *Spodoptera frugiperda* J. E. Smith is a new invasive pest in Indonesia and is reported to be invasive almost worldwide. One of the controls carried out is using synthetic pesticides. However, using synthetic pesticides causes problems, such as resistance, product and environment contamination, and environmental damage. Nanotechnology is a modern research field that has the potential to be used as an alternative pest control technology, one of which is silver nanoparticles. However, the biological synthesis of nanosilver is still limited. This research aims to determine the ability of *Bacillus thuringiensis* as a reductant in the formation of silver nanoparticles and to test its activity as an insecticide against *S. frugiperda*. The method was conducted by detecting reductant produced by *B. thuringiensis* by mixing 5 mL of 0.01 M $AgNO_3$ with 45 mL each of supernatant and bacterial pellet. The mixed solution was then shaken for 3 days at a rotation speed of 150 rpm until a color change occurred in the solution. The AgNP synthesis results were characterized using a UV-Vis spectrophotometer. The results showed that based on spectrophotometer measurements it was confirmed that AgNPs synthesized with *B. thuringiensis* reductants showed a maximum absorption peak at a wavelength of 412.9 nm. AgNPs were tested on *S. frugiperda* using various concentrations. The highest mortality values were obtained in the treatment of 10% AgNPs with larval mortality of 47%. The $LC_{50.90}$ values of 10.99% and 91.83%. The $LT_{50.90}$ values were 78.10 hours and 145,12 hours. These results indicate that bio-synthesize silver nanoparticles have the potential to be used as a bioinsecticide.

1 Introduction

The armyworm *Spodoptera frugiperda*, known as fall armyworm (FAW), originates from tropical areas such as the United States and Argentina and has spread to various countries throughout the world [1]. FAW larvae have 353 host plants from 76 plant families, are polyphagous, highly unique, and have a fast life cycle [2], attacking various plant stages [3,4]. This pest is also very difficult to control in several African countries because it has resistance to many chemical insecticides [5]. Intensive chemical insecticides can cause concerns over chronic environmental, health effects, decreasing biodiversity, and accumulating residue in the environment and plant products [6,7].

Based on this fact, its need pest management alternatives that are efficient as well as green, such as biopesticide. The progress in modern technology has provided some very promising fields one such highly innovative field is nano-technology, which can be used as a tool to transform various challenges for example development of green nanoparticles, i.e. silver nanoparticles [8]. Silver nanoparticles (AgNPs) are one of the most extensively studied types among several nanostructured materials which is interesting in nano pesticide science due to their strong insecticidal activity [9].

The application of AgNPs in pest management areas has high potential but traditional chemical and physical methods possess some limitations such as toxic, hazardous chemicals, and high energy sources [10,11]. On the other hand, biological synthesis represents a more environmentally friendly option allowing for microorganisms, plants, or biomolecules to act as reducing agents [10]. The nanoparticles are unique to the approach and can be tuned for various applications, in addition to minimizing environmental harm. The bacteria *Bacillus thuringiensis* has been an applied biocontrol agent that can be used for the synthesis process of AgNPs, and its metabolic processes assist in converting silver ions to nanoparticles [9,12].

Based on the background, research about the biosynthesis of AgNPs using *B. thuringiensis* and its toxicity to *S. frugiperda* needs to be done. This study aims to explore the potential of *B. thuringiensis* strain BT2 as a biological reductant for the synthesis of AgNPs

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and to evaluate the insecticidal activity of these nanoparticles against *S. frugiperda*. By characterizing the synthesized AgNPs and assessing their toxicity through bioassays and probit analysis, this research seeks to contribute to the development of a sustainable, effective alternative to synthetic pesticides. The findings of this study could pave the way for the broader application of biosynthesized nanoparticles in pest management, offering a more environmentally responsible solution to the challenges posed by invasive species like *S. frugiperda*.

2 Material and methods

This research was conducted at the Laboratory of Plant Protection and Environment, Study Program of Agroecotechnology, Faculty of Agriculture, Universitas Trunojoyo Madura. This research was carried out from February 2023 until November 2023.

2.1 Materials

The materials used are *B. thuringiensis* strain BT2 (isolates collection from Laboratory of Plant Protection and Environment, Faculty of Agriculture, Universitas Trunojoyo Madura), silver nitrate ($AgNO_3$), Nutrient Agar (NA), Luria Bertani (LB) broth, agar bacteriological, NaCl 0,5 M, aquadest, 300 mg Clindamycin HCl, and *S. frugiperda*'s instar 2 larvae.

2.2 Methods

2.2.1 Reculture B. thuringiensis strain BT2

B. thuringiensis strain BT2 were recultured on Nutrient Agar (NA). A total of 1 oose of bacterial isolates was streaked onto NA and stored in an incubator at 37°C for 3 days [13]. To make bacterial extracts, culture was carried out again in a 500 mL Erlenmeyer flask containing 200 mL of Luria Bertani (LB) media at 37°C and 150 rpm for 48 hours. The bacterial culture was then centrifuged at a rotation speed of 5000 rpm for 10 minutes and the supernatant was used for the synthesis of silver nanoparticles [14]. Apart from that, the pellets produced in the centrifugation process are also used for the synthesis of silver nanoparticles as a comparison for the number of nanoparticles produced. The pellet was washed twice in 0.5 M NaCl and centrifuged at 5000 rpm for 15 minutes to avoid exoprotease activity, then rinsed twice with sterile distilled water and centrifuged again. The cleaned pellets were resuspended in 100 mL of sterile distilled water and stored at 20°C until use. The pellets and supernatant obtained were used as material for the synthesis of silver nanoparticles [9].

2.2.2 Bio-synthesis silver nanoparticles

The silver nanoparticle synthesis process begins with making a stock solution of silver nitrate ($AgNO_3$) by dissolving 16.9 mg of silver nitrate in 100 mL of distilled water to obtain a stock solution concentration of 0.01 M (1.69 g L^{-1}). 45 mL of the pellet solution and supernatant from the *B. thuringiensis* strain BT2 culture were added to 5 mL of 0.01 M $AgNO_3$ solution each to obtain a total volume of 50 mL. The mixture of each supernatant and bacterial pellet with the $AgNO_3$ solution was incubated at room temperature with a rotation speed of 150 rpm for 3 days [14]. The color change in the mixture to brown is the first indicator that the nanoparticles have been successfully formed [15].

2.2.3 Characterization of silver nanoparticles

Characterization of silver nanoparticles was carried out qualitatively by observing color changes in the mixed solution between $AgNO_3$ and bacterial culture. The color change in the mixture is the first indicator that the nanoparticles have been successfully formed. The color differences depend on the size and shape of the nanoparticles [9]. The change in color of the solution is due to the reduction of silver ions to AgNP [16].

The synthesized silver nanoparticles were then characterized using a UV-Vis spectrophotometer to confirm the formation of the resulting silver nanoparticles. Silver nanoparticles have special properties, namely surface plasmon resonance (SPR). This SPR is capable of absorbing visible light at wavelengths ranging from 400-500 nm so this wavelength is used as a marker for the beginning of the formation of silver nanoparticles [17]. The UV-Vis spectrophotometer instrument was standardized using a blank in the form of *Bacillus thuringiensis* bacterial supernatant. The bacterial supernatant was placed in a cuvette and calibrated as a standard for measuring the absorbance peak of silver nanoparticles. After the calibration process is complete, the silver nanoparticle solution is put into a quartz cuvette and mounted on a spectrophotometer as a test sample. Absorbance value measurements were carried out at a wavelength of 200-800 nm.

2.2.4 Toxicity assay of AgNPs against S. frugiperda

The toxicity test of silver nanoparticles on *S. frugiperda* was carried out using the feed poisoning technique with some modification [18,19]. Each synthesized AgNP was diluted according to the concentration treatment. Fresh beans were soaked in 50 mL of solution at the concentration of each treatment for 1 hour in a petri dish and drained for 15 minutes until shade dry. Before being given treatment feed, second instar larvae of Spodoptera frugiperda obtained from the Indonesian Research Institute for Sweetener and Fiber Plants (BALITTAS) Malang, East Java, Indonesia were fasted for 12 hours. The test larvae are then transferred to a container and given treated food. Daily observations of larval mortality were observed. $LT_{50.90}$ doses of nanoparticles to kill larvae were recorded at 24, 36, 48, 60, and 72 hours after treatment. The larval mortality data was subjected to $LC_{50.90}$ probit analysis.

2.3 Experimental design and data analysis

S. frugiperda mortality data obtained was then processed using probit analysis to obtain probability values using the IBM SPSS statistics program to obtain $LC_{50.90}$ values and $LT_{50.90}$ values.

3 Result and discussion

3.1 Synthesis of silver nanoparticles (AgNPs) using *Bacillus thuringiensis* strain BT2

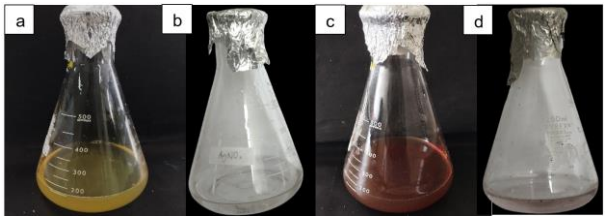

Fig 1. Different reactions of $AgNO_3$ Silver nanoparticle synthesis (a=Bt supernatant without adding $AgNO_3$ (no reaction), b=Bt pellet without adding $AgNO_3$ (no reaction), c=Bt supernatant with adding AgNO3 (positive reaction with change color), d=Bt pellet with adding AgNO3 (no reaction).

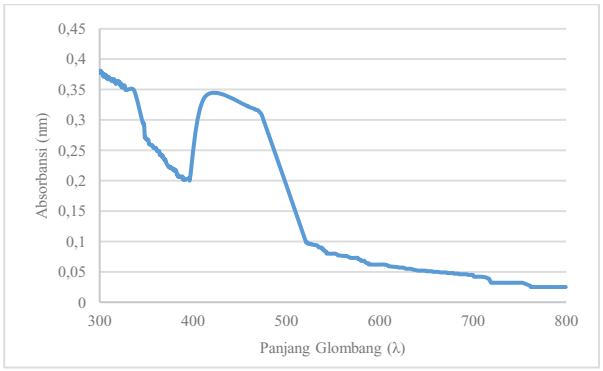

Fig 2. The absorption spectrum of silver nanoparticles synthesized by *B. thuringiensis* strain BT2 supernatant.

Based on Fig 1, it can be seen that the $AgNO_3$ solution which has been added with bacterial supernatant shows a color change from yellow to brown, while $AgNO_3$ with the addition of bacterial pellets, $AgNO_3$ solution without the addition of bacterial pellets and bacterial supernatant without the addition of $AgNO_3$ do not show a color change. The color change in the mixed solution of bacterial supernatant with $AgNO_3$ indicates that silver nanoparticles are produced extracellularly. This provides an advantage in the silver nanoparticle production process because the reducing agent or reducing agent can be obtained extracellularly, making it easier to harvest the reducing agent.

The silver nanoparticles that had been formed from the bacterial supernatant were then characterized using a UV-Vis spectrophotometer to confirm the formation of the resulting silver nanoparticles. Based on the results of analysis using a UV-Vis spectrophotometer, AgNPs produced from the biosynthesis using *B. thuringiensis* supernatant obtained the highest absorbance appearance at 412.9 nm which shows an absorbance value of 0.338 (Fig. 2). This wavelength is close with other study showed that the absorption peak was found at 420 nm [20]. Similar to other AgNP bioreduction, *B. firmus*, at 425 nm [21]. The absorption peak at a wavelength between 400-500 is the initial marker for the formation of silver nanoparticles [22].

3.2 Silver nanoparticles (AgNPs) toxicity test against *S. frugiperda*

The mortality value of *S. frugiperda* larvae showed the highest value in 10% AgNP treatment with a mortality of 47%. These data show that the higher the concentration of silver nanoparticles used, the percentage of larval mortality also shows an increase (Table 1). The effect of silver nanoparticle poisoning on *S. frugiperda* shows symptoms in the form of a blackish color change due to silver nanoparticle poisoning (Fig. 3). Previous study reported that AgNP toxicity tests against *Spodoptera litura* and *Achaea janata* showed that the accumulation of Ag in the rough endoplasmic reticulum and other cell organelles such as the nucleolus and mitochondria resulted in physiological changes in the larval body and caused oxidative stress in cell organelles [23–26].

Table 1. Larval mortality is caused by AgNPs at 72 hours after treatment.

Treatment	Larvae death (larvae)	Mortality (%)
0% AgNP	0	0
0.5% AgNP	1	3
1% AgNP	2	7
5% AgNP	10	33
10% AgNP.	14	47

Fig 3. Comparison larval changes after 72 hours between control (a) and treatment using AgNPs (b).

The $LC_{50.90}$ value is the value used to determine the concentration of silver nanoparticles which are capable of killing 50% and 90% of the total test larvae. The following is a table of $LC_{50.90}$ probit analysis results for AgNP (See Table 2). Data from probit analysis of $LC_{50.90}$ AgNP shows that to kill 50% of larvae is required 10.991% AgNP, while to kill 90% of larvae is required 91.826% AgNP. The results of the probit analysis show that there is a linear model of the relationship between concentration and mortality with the equation Y= a+bX, with the a value being -1.45 and the b value being 1.39, so that the regression equation

Y= (-1.45)+1.39X with R^2=0.997, which means that the effect of giving silver nanoparticles in killing the test larvae was 99.7%. The closer the R-value approaches 1, the stronger the influence of log concentration (X) on mortality (Y).

The $LT5_{0.90}$ value is a reference value used to determine the period needed to kill 50% and 90% of the total test larvae. $LT_{50.90}$ is calculated using a concentration close to LC_{50}, namely a concentration of 10% (Table 2). The results of probit analysis at 10% AgNP showed that killing 50% of the total larvae took 78.097 hours while killing 90% of the total larvae needed 145,119 hours (Table 3). The linear regression equation Y=a+bX shows that the a value is -6.79 and the b value is 3.54, so the regression equation is Y=(-6.79)+ 3.54X with the R^2 value being 99.7%. The closer the R^2 value approaches 1, the stronger the influence between log concentration (X) and mortality (Y).

Table 2. Probit analysis results for $LC_{50.90.}$

Mortality (%)	Estimation of concentration probability (%)
50	10.991
55	13.534
60	16.722
65	20.807
70	26.198
75	33.593
80	44.308
85	61.183
90	91.826

Table 3. Probit analysis results for $LT_{50.90}$ at 10% concentration.

Mortality (%)	Estimation of time probability (hours)
50	78.097
55	82.989
60	88.274
65	94.090
70	100.634
75	108.208
80	117.315
85	128.901
90	145.119

Based on the data, the research findings on the biosynthesis of silver nanoparticles (AgNPs) using *B. thuringiensis* strain BT2 demonstrate promising potential for insecticide, especially in combating S. frugiperda. This study revealed that the silver nanoparticles were produced extracellularly, confirmed by a color change in the solution, and characterized by UV-Vis spectrophotometry showing a maximum absorption peak at 412.9 nm. This indicates successful nanoparticle formation, comparable to previous studies. The biological synthesis approach is significant because it eliminates the need for toxic chemicals typically involved in nanoparticle production, offering a green and more sustainable method. Additionally, *B. thuringiensis*, a known biocontrol agent, plays a dual role here as both a biopesticide [27,28] and a nanoparticle synthesizer.

The insecticidal efficacy of the biosynthesized AgNPs against *S. frugiperda* larvae was tested across different concentrations, with the 10% AgNP treatment resulting in 47% larval mortality. The probit analysis revealed LC_{50} and LC_{90} values of 10.99% and 91.83%, respectively, showcasing the dose-dependent toxicity of AgNPs. This concentration was more effective than herb extract [19]. The LT_{50} value of 78.10 hours at a 10% concentration further supports the potential of AgNPs as an efficient bioinsecticide. The mechanism behind this toxicity likely stems from the oxidative stress and cellular damage caused by silver nanoparticle accumulation, as observed in previous studies on related pests [23]. This research provides a foundation for the development of nanoparticle-based biopesticides that are both effective and environmentally responsible. Such innovations are crucial in the global effort to manage invasive pests while reducing the reliance on harmful chemical insecticides.

4 Conclusion

Silver nanoparticles (AgNPs) can be synthesized biologically using *B. thuringiensis* strain BT2 as a reduction agent. SPR analysis shows the highest absorbance of AgNP at a wavelength of 412.9 nm with an absorbance value of 0.338. The AgNPs synthesized using *B. thuringiensis* strain BT2 have a toxic effect that can kill *S. frugiperda* larvae with a mortality value of 10% of 47% with an $LC_{50.90}$ value of 10.99% and 91.83%. The $LT_{50.90}$ value at 10% AgNPs showed 78.10 hours and 145.12 hours. AgNPs synthesized using *B. thuringiensis* strain BT2 had the potential as a bioinsecticide.

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