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Effectiveness of *Samia cynthia ricini* Boisduval (Lepidoptera: Saturniidae) Cocoon Extract as UV Protectant of *Bacillus thuringiensis kurstaki* in Controlling Beet Armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) under Sunlight

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ABSTRACT

Bacillus thuringiensis (Bt) is a biological agent for insect pest management. Its toxins effectively control *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae, but it is sensitive to ultraviolet (UV) radiation from the sunlight. This study aimed to investigate the effect of sericin extract from *Samia ricini* Boisduval (Lepidoptera: Saturniidae) cocoons as a UV protectant for Bt after exposure under direct sunlight for 1, 2, 3, and 4 weeks. After being exposed to sunlight, the Bt formulae were tested against 20 larvae of 24 hr old, the first larval instar in the laboratory. The larval mortality was observed 72 hr after the treatment. The results indicated that the mortality of *S. exigua* in Bt + sericin extract treatment was significantly different compared with Bt alone. For the first week, the mortality of *S. exigua* in exposed Bt + sericin exposed Bt alone, unexposed (Bt + sericin, and unexposed Bt alone were 80, 61, 85, and 97%, respectively. Scanning electron microscopy analysis revealed that Bt + sericin, after being exposed to sunlight, still showed the presence of spore and crystal protein comparable to the unexposed Bt.

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Based on the results, sericin provides good protection against sunlight and prevents the Bt spores from light-induced damage.

Keywords: Bt, *Samia*, *Spodoptera exigua*, UV protectant

INTRODUCTION

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is a polyphagous insect pest that can damage many plants, such as wheat, cotton, pea, cauliflower, tomato, onion, and soybean (Adamczyk et al., 2008; Saeed et al., 2010; Taylor & Riley, 2008; Zheng et al., 2000; Zhou et al., 2011). The larvae can feed on leaves and attack flowers, buds, and fruits, causing slow growth and a decrease quantity and quality of the crops (Khattab, 2013). Thus, controlling the insect pest population is critical, thereby promoting the use of chemical and biological insecticides (Meissle et al., 2011). Chemical pesticide reliance results in increased insecticide resistance, a greater risk to human health because of the lack of appropriate safety standards, and environmental pollution (Day et al., 2017). *Bacillus thuringiensis* (Bt) is an alternative solution for reducing insect pests. With a narrow species spectrum, these bioinsecticides are safe, eco-friendly, residual-free, and safe for most other organisms (Maagd et al., 2001; Schnepf et al., 1998).

Bt is a Gram-positive bacterium that can produce toxic proteins (para-spore crystals) when reaching the sporulation stage (Bravo et al., 2005). These insecticidal crystal proteins are predominantly encoded by *Cry* and *Cyt* genes, known as δ-endotoxins (Aronson, 2002). When insect larvae ingest such toxins during feeding, spore, and toxins (δ-endotoxins) are produced in the midgut. These molecules (Cry and Cyt) bind to receptors on the intestinal epithelium, causing irreversible damage to the epithelial cells by generating pores or lysing the target membrane of the midgut (Gill, 1995). Cry proteins show a highly specific spectrum and effectiveness in insect taxa, such as Diptera, Lepidoptera, Coleoptera, and Hymenoptera (Maagd et al., 2001). In addition, Cry1C is a major specificity determinant for *S. exigua* (Maagd et al., 2000). However, biological pesticides are easily degradable and unstable when applied in the field. For example, sunlight's ultraviolet (UV) radiation might deactivate the Bt toxin (Sansinenea et al., 2015). Therefore, treatment measures are necessary to address this difficulty.

Recently, scientists tried to improve the stability of bio-pesticides by covering Bt spores and toxins using biomaterials as UV protectants. For example, *Bacillus thuringiensis kurstaki* (Btk) was formulated with a biomaterial derived from olive oil (Maghsoudi & Jalali, 2017), tea leaves (Ningrum & Sumarmi, 2020), aloe vera (Tarigan et al., 2020), spinach leaves (Sumarmi et al., 2020), and sericin extract from eri and atlas silkworm (Sukirno et al., 2022). Silk sericin is a biological polymer consisting of glycoprotein, essential for protecting the cocoon and pupa from UV radiation during pupal stages (Kaur et al., 2013). It also has antioxidant activity that can protect against UV radiation and overcome oxidation (Kumar & Mandal, 2019).

Recently, sericin from eri and atlas cocoon has been proven effective as a UV protectant of Btk (Sukirno et al., 2022) against tobacco armyworm. This study evaluated the effectiveness of formulation of Btk and sericin extract of eri silkworm cocoon when sunlight against beet armyworm. In addition, the potency and viability of Btk spore crystals with the addition of sericin extract were evaluated in the laboratory. The results of this work will support the integrated management of insect pests in Indonesia.

MATERIALS AND METHODS

Beet Armyworm Insect Rearing

Beet armyworm larvae were collected from onion farms in Magelang, Central Java, Indonesia. The larvae were maintained in the lab using an artificial diet (Shorey & Hale, 1965) with some modifications (Sukirno et al., 2018) until pupation. Thirty pupae were each transferred in a glass jar (7 cm × 23 cm) with an opaque paper supporting adult emergence, mating, and egg laying. Adults were provided with a cotton ball dipped in a 10% honey solution for feeding. Eggs were monitored and collected daily, then kept in plastic cups (6.5 cm × 4 cm) containing an artificial diet and then covered with tissue until hatched into first instar larvae. Insects were kept in lab conditions of 28 ± 5°C and relative humidity of 50 – 70%. A 24 hr old first larval instar of F_2 was used in this study.

Bio-pesticide and Sericin Extract Preparations

Dipel WP®, a commercial product by Abbot Co. (Indonesia), was used as a source of *B. thuringiensis kurstaki*, whereas eri silkworm cocoon was collected from Jantra Mas Sejahtera (JAMTRA, Indonesia) wild silk production house. Five grams of dried cocoon was cut into small pieces and added into 100 mL of autoclaved distilled water (dH_2O) containing 1 g of Turkey Red Oil (TRO, Indonesia) powder. Then, the mixture was boiled for 60 min using a stirring machine and filtered to obtain a stock of 5% sericin solution. Before the experiment, the stock solution was diluted using autoclaved dH_2O to make a 1% sericin solution.

Larval Toxicity Bioassay

The 1% sericin extract was added to make a Bt suspension at 10 times the lethal concentration of 95% ($LC_{95)}$ (2×10^8 CFU/ml) (Sukirno et al., 2022). Autoclaved dH_2O as a negative control was used to compare the effectiveness of UV protectants. One milliliter of each Bt suspension was transferred homogenously to the surface of the disposable Petri dish (55 mm × 15 mm). After that, the Petri dishes were exposed to direct sunlight for 1, 2, 3, and 4 weeks. After respective exposure, the dried Bt was then homogenously mixed with 10 ml of autoclaved dH_2O to make the final concentration at LC_{95}. Afterward, 1 ml of each formula was poured onto an artificial diet surface in a plastic cup (6.5 cm × 4 cm). The Bt-contaminated diet was allowed at room temperature for 2 hr before adding the 24 hr old first larval instar of *S. exigua*. The experiment used five replicates on each formula and exposure period, with 20 larvae per replicate, for a total of 2,000 treated larvae. The mortality was recorded at 72 hr after treatment and scored as dead when there was no movement when touched with a fine brush. In this study, the first larval instar of beet armyworm was used as an indicator for the effectivity of UV protectant of Bt as it is susceptible.

Sublethal Effects of Bt Formulation on Beet Armyworm Larvae

Sublethal effects can be physiological, behavioral, survival rate, and body weight after treatment with sublethal amounts of toxic compounds (Desneux et al., 2007; Rajathi et al., 2010; Tao & Wu, 2006). The sublethal effects of Bt on the larval body weight beet armyworm in the present were observed on the sixth day after the treatment.

Scanning Electron Microscopic Observation of Bt Formulation

Scanning electron microscopy (SEM) (JSM-6510LA, JEOL Ltd., Japan) was used to evaluate the presence and the structure of spores and crystals. One milliliter of Btk suspension was centrifuged at 16,000 × *g* for 15 min at 4°C and air dried on a castable vacuum system. Samples were then placed on a brass stub, sputter coated with gold at 3.3 Pa and 20 mA for 120 s, and photographed in a JEC-3000 FC (JEOL Ltd., Japan) at 15 kV.

Statistical Analysis

Before statistical analysis, the data on mortality percentage was corrected using the Abbott formula (Abbott, 1925; Finney, 1977). Then, the mortality percentage and larval weight were analyzed using a one-way analysis of variance (ANOVA). The exposure period and formula were used as independent factors, with mortality and larval weight as dependent factors for analysis. Before the ANOVA, the mortality percentage was arcsine-transformed. After the ANOVA, the means were subjected to post hoc multiple pairwise comparisons across each formula and exposure period ($P < 0.05$; least significant difference [LSD]). Statistical Product and Service Solutions (SPSS) Statistic (ver. 23.0) was used for all statistical analyses.

RESULTS

Mortality of the First Instar Larvae of *S. exigua*

The percentage of larval mortality was significantly different for each formula during the first week of exposure ($F_{4,20} = 12.75$; $P = 0.000$) and the fourth week of exposure ($F_{4,20} = 9.14$; $P = 0.001$). Meanwhile, no statistically significant differences were identified after the second week of exposure ($F_{4,20} = 2.75$; $P = 0.077$), the third week of exposure ($F_{4,20} = 0.36$; $P = 0.782$), and without sunlight exposure ($F_{4,20} = 2.11$; $P = 0.139$).

In the first week, the pathogenicity of the Bt + sericin had higher toxicity against beet armyworm larvae (80%) than the Bt without sericin (61%). By contrast, Bt +

sericin and Bt without sericin in unexposed sunlight had relatively higher pathogenicity to *S. exigua* larvae, with mortality of 85 and 97%, respectively. It showed that the formula of Bt mixed with sericin was more pathogenic to *S. exigua* than Bt alone when exposed under sunlight, with values ranging from 56 to 93%, except on the second week of exposure. The pathogenicity value of the combination of Bt + sericin without exposure to *S. exigua* ranged from 85 to 98%, whereas that of Bt alone without exposure ranged from 81 to 99%. The death rate showed a synergistic effect of sericin when added to Bt and tested against beet armyworm (Table 1).

Delayed Mortality and Sublethal Effects on the First Instar Larvae of *S. exigua*

Figure 1 shows that the percentage of surviving larvae during the exposure period until the sixth day was not significantly different in all treatments. The percentages were 3.97, 3.97, 1.48, and 0.72% ($F_{3,12}$ = 1.36; P = 0.300) for exposed Bt + sericin, exposed Bt without sericin, unexposed Bt + sericin, and unexposed Bt without sericin, respectively. The average larval weight in Bt + sericin is greater than that of exposed Bt without sericin, unexposed Bt + sericin, and unexposed Bt without sericin treatments (Figure 2). The larval weight on those treatments was 1.67; 0.93; 0.29, and 1.00 mg ($F_{3,12}$ = 0.37; P = 0.774), respectively. Meanwhile, the larval weight during the 2-week exposure period was significantly different and higher than other exposure periods, whereas 1, 2, 3, and 4 weeks was 0.38, 3.27, 0.20, and 0.03 mg ($F_{3,12}$ = 7.05; P = 0.005), respectively. On the other hand, based on the larvae survivorship, it showed that there was no significant difference

Table 1
Mortality percentage of the first instar larvae of Spodoptera exigua *(mean ± SE) after being treated with* Bacillus thuringiensis kurstaki *formulation at different exposure periods*

Period (Week)	Mortality exposed (%)		Mortality non-exposed (%)		Statistic
	Bt + sericin	Bt without sericin	Bt + sericin	Bt without sericin	
0	93.63 ± 2.31aA	82.60 ± 6.51aA	96.35 ± 0.92aA	93.04 ± 3.39aAB	$F_{4,20}$ = 2.11; P = 0.139
1	80.00 ± 4.18abA	61.14 ± 7.08aB	85.00 ± 3.53bA	97.14 ± 2.85cB	$F_{4,20}$ = 12.75; P = 0.000
2	56.00 ± 13.72aB	86.33 ± 5.71aA	93.78 ± 5.06aA	81.76 ± 5.02aA	$F_{4,20}$ = 2.75; P = 0.077
3	91.04 ± 5.57aA	87.00 ± 4.89aA	89.00 ± 8.57aA	96.04 ± 0.98aAB	$F_{4,20}$ = 0.36; P = 0.782
4	93.09 ± 2.52abA	86.00 ± 1.87aA	98.00 ± 1.22bA	99.04 ± 0.95bB	$F_{4,20}$ = 9.14; P = 0.001
Statistic	$F_{4,20}$ = 3.34; P = 0.030	$F_{4,20}$ = 2.99; P = 0.044	$F_{4,20}$ = 1.73; P = 0.183	$F_{4,20}$ = 3.37; P = 0.029	

Note. Means with the same row followed by the same lowercase letters are not significantly different at $P < 0.05$. The numbers with the same column followed by the same uppercase letters are not significantly different at $P < 0.05$

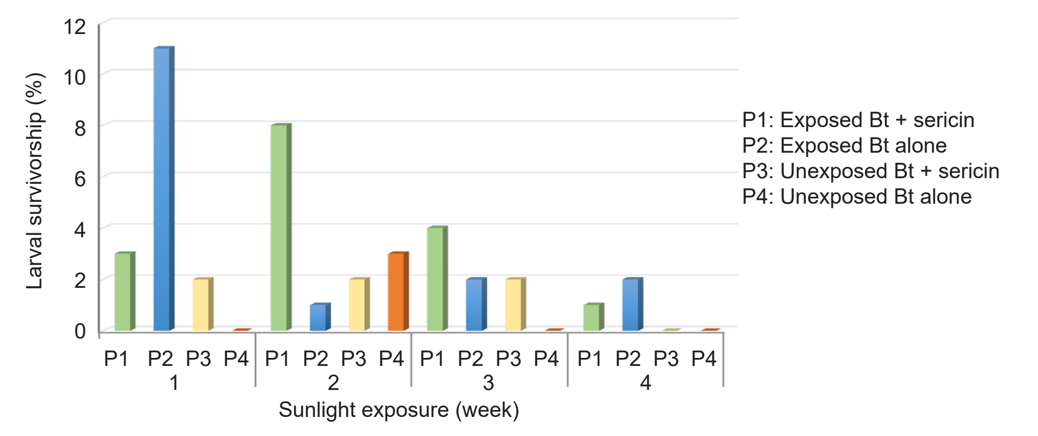

Figure 1. Effects of different Bt formulations on the average number of *Spodoptera exigua* survival on the sixth day after treatment

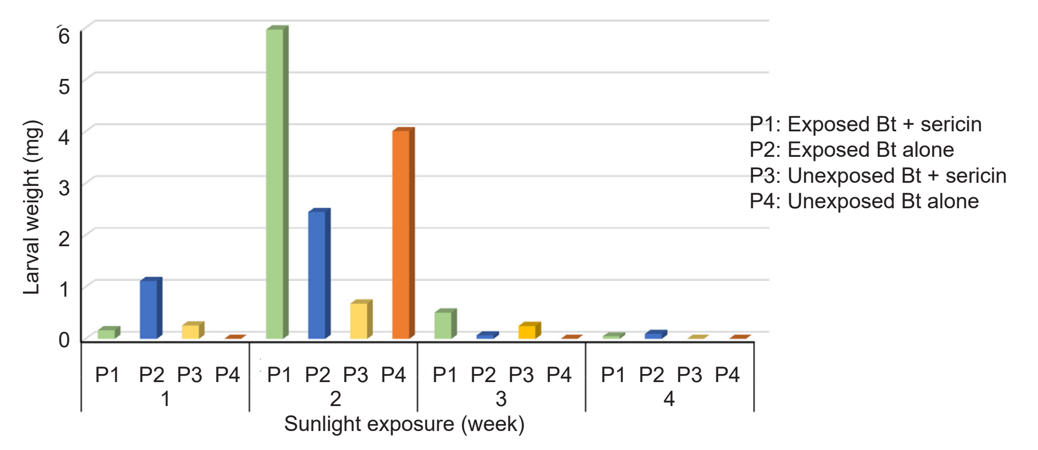

Figure 2. Sublethal effects of different Bt formulations on the larval weight of *Spodoptera exigua* on the sixth day after treatment

in 1-, 2-, 3-, and 4-week treatment. The survivorship on those treatments was 3.97, 3.45, 1.98, and 0.74% ($F_{3,12}$ = 0.94; P = 0.448), respectively.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) ((JSM-6510LA, JEOL Ltd., Japan) has successfully detected the spore and crystal of Bt in the treatment, especially on the formula with and without exposure to direct sunlight. This research found many spores within various crystal shapes, including bipyramidal, cuboidal, and spherical crystals (Figures 3 and 4).

DISCUSSION

The protein sericin content in silkworm cocoons is lower than fibroin, around 20–30% (Eom et al., 2020). Sericin is rich in

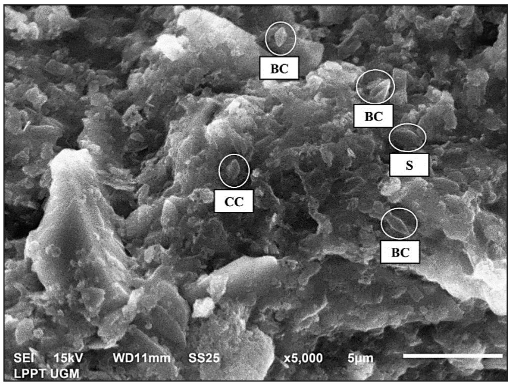

Figure 3. Scanning electron microscopy of the mixture of Bt spores, crystals, and sericin extract (1%) without exposure
Note. S = Spore; CC = Cuboid crystals; BC = Bipyramidal crystal

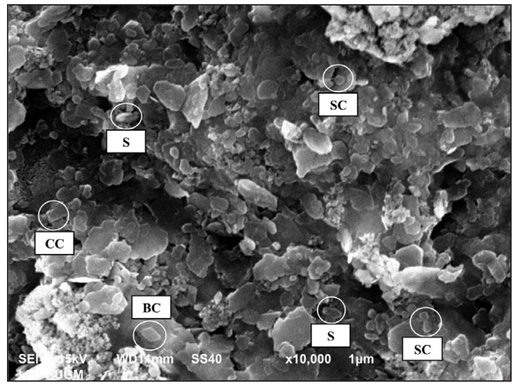

Figure 4. Scanning electron microscopy of the mixture of Bt spores, crystals, and sericin extract (1%) after three weeks of exposure
Note. S = Spore; CC = Cuboid crystals; BC = Bipyramidal crystal; SC = Spherical crystal

amino acids, particularly serine and aspartic acid, and each cocoon layer has different sericin contents (Aramwit et al., 2010). Several studies have shown that sericin has a high concentration of antioxidant molecules, which play an important role in preventing UV-induced oxidative damage (Kaur et al., 2013). A previous study has demonstrated that sericin from eri silkworm can absorb UVA and UVB radiation and has a high toxicity effect on *Spodoptera litura* larvae when mixed with Btk for bioassay (Sukirno et al., 2022). UV radiation is an electromagnetic spectrum that is categorized into three regions: 320–400 nm as ultraviolet A (UVA), 280–320 nm as ultraviolet B (UVB), and 200–280 nm as ultraviolet C (UVC). UVA accounts for 90–98% of total UV radiation reaching the earth, whereas UVB accounts for 1–10%. Moreover, UVC radiation is absorbed by the ozone layer (Hou et al., 2015; Kaur et al., 2013).

We evaluated a small concentration (1%) of sericin extract as UV protectant Bt under direct sunlight exposure in the present study. The results showed that sericin significantly affected the persistence of Bt to prolonged time exposure, as revealed by the mortality percentage of *S. exigua* larvae, which was greater in Bt with sericin than in Bt alone (Table 1). This extensive research also tested the efficacy and survivability of Bt spore crystals. SEM analysis revealed that after prolonged exposure to the formula with the addition of sericin extract (1%), spores and crystals were still observed despite being exposed to sunlight for three weeks. Many spores within various crystal shapes were found (Figures 3 and 4), which included bipyramidal, cuboidal, and spherical crystals. These results are consistent with the research results of Lozano et al. (2018), who discovered the crystal shape of Btk S-1905. Larvicidal toxicity was commonly due to spores and crystal proteins. The Cry 1 protein, which is efficient against Lepidoptera, is associated with bipyramidal crystals, whereas the Cry 2 protein, which is effective against Diptera and Lepidoptera, is associated with cuboidal crystals (Silva et al., 2004). Other factors, including the structure and function of the intestine, toxin diversity, structure and solubilization of protein, and toxin interactions, were also investigated (Gill, 1995). The presence of spores and germination can affect Bt insecticidal activity (Liu et al., 1998). The spores can be protected by various localized biological structures, such as spore coats on Bt, allowing crystal formation to continue during sporulation. Spores of *Bacillus* species are seven to 50 times more resistant to UV radiation at 254 nm, the wavelength that kills most cells, than vegetative cell killing (Setlow et al., 1998). Furthermore, according to Hart et al. (2006), spores have an outer layer known as an exosporium, which surrounds the dense spore and serves as a protective barrier. This layer is present in some spore species, such as *Bacillus anthracis* and *Bacillus thuringiensis*, but absent in others.

Larval body weight as an indicator of sublethal effects was significantly related to the proportion of surviving larvae, which led to major changes in the biology of the treated larvae and their offspring. The results showed that the Bt formulation with sericin extract after the fourth week of exposure decreased the proportion of survivors. In addition, the larval weight emergence rate was significantly reduced on the sixth day after treatment for all formulas. The percentage of larval survival in P1 and P2 formulas had the same value but not with their body weight. Our hypotheses indicate that differences in survival ability can be observed among larvae, with a high adaptation to diet contamination. Another factor may be due to the different counts of spore present in each formula. According to Apaydin et al. (2008), almost all strains of Bt suppress the growth and development of the larvae, which might cause pupal stage failure.

CONCLUSION

The study showed that sericin (1%) from cocoons of *S. cynthia ricini* protected Bt from exposure to direct sunlight and that the formula was efficient against *S. exigua* larvae. In addition, the mortality percentage, sublethal effect, and existence of spores and crystals indicated the efficacy of the treatment. However, all bioassay processes are still conducted on a laboratory scale; thus, further studies may be required to evaluate their protective effectiveness directly in field conditions, particularly on onion crops.

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