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Research Article

Attacus atlas (L.) sericin extract as an effective UV Protectant of *Bacillus thuringiensis* serotype *kurstaki* for controlling *Spodoptera litura* (Fab.)

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ABSTRACT

Bacillus thuringiensis serotype *kurstaki* is an entomopathogenic bacteria commonly used to control the cutworm *Spodoptera litura* (Fab.). However, *B. thuringiensis* has disadvantage of being easily degraded due to sunlight. The objective of this research was to determine the effectiveness of adding *A. atlas* (L.) cocoon extract as UV protectant *B. thuringiensis* to the mortality of *S. litura*. This research formulated 2.5% of the original substance of *A. atlas* cocoon extract and *B. thuringiensis* serotype *kurstaki* strain HD-7 applied from commercial product DiPel-WP®. The formulation was exposed to sunlight for 0, 1, 2, and 3 weeks. The suspension treated for 20 individuals of first instar larvae *S. litura* shifted into the artificial diet using 3-5 replicates. The scanning electron microscope (SEM) method began from a sample that was vacuumed, sample coated, and observed on SEM with the electron in a certain level probe. This research showed that the mortality of *S. litura* decreased with the growth of *S. litura*. The mortality of *S. litura* achieved 20-100% mortality after treatments. The *A. atlas* cocoon extract was effective as UV protectant *B. thuringiensis* for three weeks of exposure to sunlight. The SEM analysis represented that formulation of *B. thuringiensis* and *A. atlas* cocoon extract with sunlight exposure for one week has more rough surface than that of exposed during three weeks.

INTRODUCTION

Spodoptera litura (Fab.), known as armyworm is considered one of the major pests of crops. It attacks 27 species of crops from 24 genera, 14 families divided into several ranges of plants, comprising vegetables, weeds, fruits, and ornamental plants (Ahmad et al. 2013). For example, *S. litura* threatens the production of soybeans up to 80%, thus affecting cultivation. It may also attack tobacco and cause 57% loss of crop yields during the dry season (BPTD 2011). It considers a polyphagous insect, thus, they able to adapt to an unstable environment (Kennedy & Storer 2000).

The chemical pesticide commonly used to control *S. litura* led to insect resistance (Vengateswari et al. 2020) and toxic residues in the environment and crop products. For example, pyrethroid and organophosphate applied in insecticide treatments for *S. litura* reported the most sig-

nificant resistance (Ahmad et al. 2013). To overcome these problems, one of the biological agents commonly used for *S. litura* control is *B. thuringiensis* (Lacey et al. 2001) . *B. thuringiensis* has crystal protein that can be toxic for many larvae of insects (Baum et al. 1999), called as cry toxins (Glare et al. 2017). It can be used as a pest management component (Obeidat et al. 2004; Glare et al. 2017). It is known that *B. thuringiensis* is safe for non-target organisms. But, it deteriorated rapidly in an environment (Khetan 2001) due to ultraviolet exposure from sunlight (Cohen et al. 1991).

Attacus atlas cocoon extract has sericin and fibroin potential as UV protectants for *B. thuringiensis* (Roy et al. 2012). Sukirno et al. (2021) found that 2.5% of *A. atlas* cocoon extract effectively protects *B. thuringiensis* for up to 4 weeks under UV B treatment. The UV spectrophotometry analysis showed that *A. atlas* cocoon extract was able to absorb UV-C (200-280 nm), UV-B (280-320 nm), and UV-A (320-400 nm). Therefore, this research was conducted to study the effectiveness of sericin from *A. atlas* cocoon extract as UV protection for *B. thuringiensis* exposed to sunlight based on the mortality of *S. litura*. The formulation of sericin and *B. thuringiensis* was observed by SEM (Scanning Electron Microscope).

MATERIALS AND METHODS

Materials

Attacus atlas cocoon was collected from rearing at Entomology Laboratory and fed on baringtonia leave (*Baringtonia asiatica* Kurzt.). A total of 1,600 individuals' larvae first instar of *S. litura* were treated by *B. thuringiensis*. TRO (Turkish Red Oil) for making extract solution. *B. thuringiensis* DiPel-WP® serotype *kurstaki* strain HD-7. Pure honey (Madu Nusantara®, PT. Madu Murni Nusantara, IN) for adult feeding and the ingredient of artificial diet used in this study is presented in Table 1.

Table 1. The compositions of an artificial diet for larvae of *S. litura*

Ingredients	Total amount
White beans (g)	250
dH_2O (ml)	1,200
Agar powder (g)	50
Benzoic acid (g)	10
Yeast (g)	80
Ascorbic acid (g)	20

Methods

This research was conducted at Entomology Laboratory, Faculty of Biology Universitas Gadjah Mada from October 2021-February 2022.

Collecting and Rearing of the Insects

Spodoptera litura was collected from cabbage, onion, and cauliflower at Sengi, Dukun, Magelang Central Java (7°31'41.8"S 110°21'06.4"E). The rearing was conducted at the Entomology laboratory with temperatures of about 27 + 33°C and 70 + 75% relative humidity (r.h.). An artificial diet for *S. litura* was made based on Sukirno et al. (2021). The composition of artificial diet for larvae of *S. litura* is shown in Table 1. Firstly, larvae of *S. litura* were placed in a plastic cup (70 ml) containing 15 ml of artificial diet until pupae. Pupae were collected daily and kept until emerging in a glass jar. Ten percent of honey solution was used for moth feeding. The folded opaque paper was put in the middle of a jar for oviposition.

Extraction of *A. atlas* Cocoon

A. atlas extraction was using the alkaline lysis method. TRO (Turkish Red Oil) was used to make 5% sericin solution. As much as 15 g of *A. atlas* cocoon and 2 g TRO was added with up to 300 ml dH_2O and heated for 60 minutes at 100°C. The 2.5% solution of *A. atlas* cocoon made using 75 ml was taken from the stock solution, and dH_2O was added to 150 ml. The suspension was kept at 4°C until further used.

Preparation of *B. thuringiensis* suspension

Bacillus thuringiensis commercial formulation DiPel-WP® serotype *kurstaki* strain HD-7 (Abbot Co., IN) mixed with the cocoon extract solution (1 g/10 ml of dH_2O). Then, it homogenized using a vortex for several minutes.

SEM (Scanning Electron Microscope) analysis of *B. thuringiensis* formulation

The first procedure took the suspension using a micropipette on carbon tape in the specimen holder and put it into a castable vacuum until it dried. The second step is Au coated procedure. Then, the sample was put in an auto coater and waited for vacuum coater until the pressure was on +3.2 Pa, then it started to coat for +120 seconds. Thirdly, observation of sample used the SEM method. The sample from the suspension then put in SEM and vacuumed for +-60 seconds, the sample electron shot by certainly probe level. The last step observed the topography of the sample surface.

The effect of sunlight on the pathogenicity of *B. thuringiensis* against *S. litura*

The bioassay was carried out using four treatments with three to five replicates, and each replication used twenty larvae. In Table 2, the treatments were applied 1 ml formulation of *B. thuringiensis* and *A. atlas* cocoon extract into a disposable petri dish, the cover of the petri dish was wrapped tightly using parafilm, and exposed to sunlight. After exposure, the suspension was diluted with 10 ml autoclaved dH_2O. The diluted suspension (1 ml) was taken to an artificial diet and left air dried for 2 hours under lab conditions, then it was tested against 20 individuals' first instar larvae of *S. litura*.

Table 2. Treatments of the Bt. Formulations under sunlight exposures

Code	Category	Treatments
P1	Exposure	*B. thuringiensis* added with cocoon extract
P2	Exposure	*B. thuringiensis* without cocoon extract
P3	No Exposure	*B. thuringiensis* added with cocoon extract
P4	No Exposure	*B. thuringiensis* without cocoon extract

Notes; (-): no cocoon extract; (+): add cocoon extract

Experimental design and Statistical analysis

Mortality of *S. litura* was recorded on the first, third, and seventh days after treatment (DAT). The percentage of increased mortality of *S. litura* on the third day was analyzed using analysis of variance (ANOVA) at α: 0.05 and post hoc analysis using Tukey HSD. All the statistical procedures were using IBM SPSS 23. Analysis *B. thuringiensis* used SEM was analyzed descriptively.

RESULTS AND DISCUSSION

The effect of sunlight on the pathogenicity of *B. thuringiensis* against *S. litura*

This research was used to treat the first instar larvae of *S. litura*. as they were more sensitive than other instars. Blouch et al. (2020) and Hallad et al. (2011) reported that the increasing of *S. litura* age in larvae resulted in the decreasing of the larval mortality. The larvae growth was followed by physiological resistance to the toxin, thus declining the ability of the toxins to bind in the midgut epithelium.

The mortality of *S. litura* after treated with *Bt.* is shown in Figure 1. Exposure to W-1 to W-3 increased the mortality of *S. litura* from the first day until the seventh day. Moreover, exposure to W-0 percentage of mortality increased from the first day until the third day. On seventh-day mortality, it was not increased due to the mortality rate approaching 100%. *B. thuringiensis* toxins need few hours to dissolve and to interact with the epithelium of midgut (Song et al. 2016). It required 8 hours to activate the toxin at midgut and caused cell apoptosis (e Castro et al. 2019).

Table 3 present the R^2 and the regression equations. Statistical analysis showed that there was no significant correlation between variable of sunlight exposure periods and mortality on the first day of mortality $F_{2,57} = 0.97$; $P>0.005$. On the third day of observation, the mortality was a statistically significant difference between variable exposure and mortality $F_{2,57} = 45.14$, $P<0.005$, and on the seventh day of mortality, there was a statistically significant relationship between variable exposure and mortality $F_{2,57} = 19.51$, $P<0.005$. The highest value R^2 value is

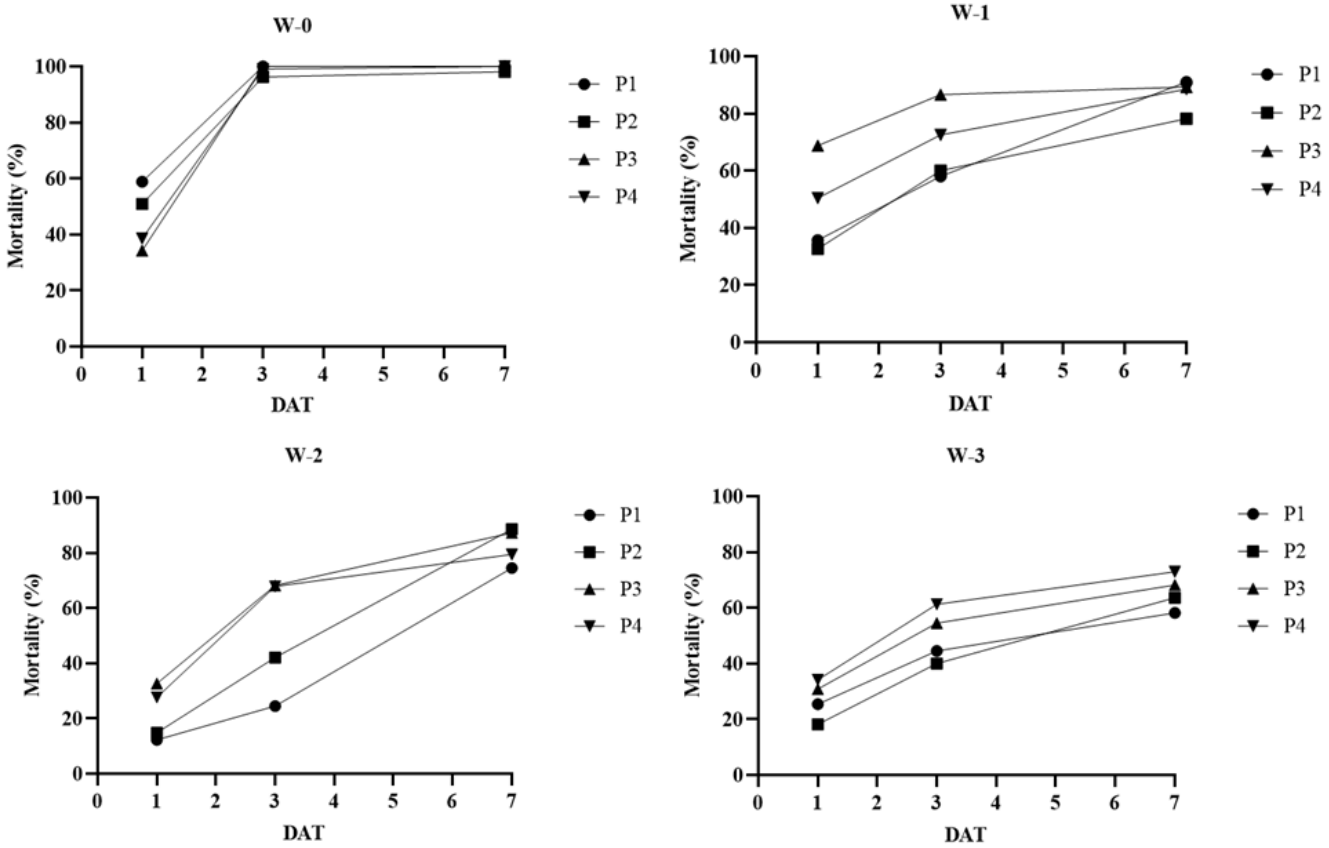

Figure 1. The effect of sunlight on the pathogenicity of *B. thuringiensis* against *S. litura*.
Note: DAT (day after treatment) W-0 (0 week); W-1(one week); W-2 (two weeks), and W-3 (three weeks). Mortality was recorded on the first, third, and seventh days after being treated with P1 (exposed and added cocoon extract); P2 (exposed and without cocoon extract); P3 (no exposed and added cocoon extract); P4 (no exposed and without cocoon extract).

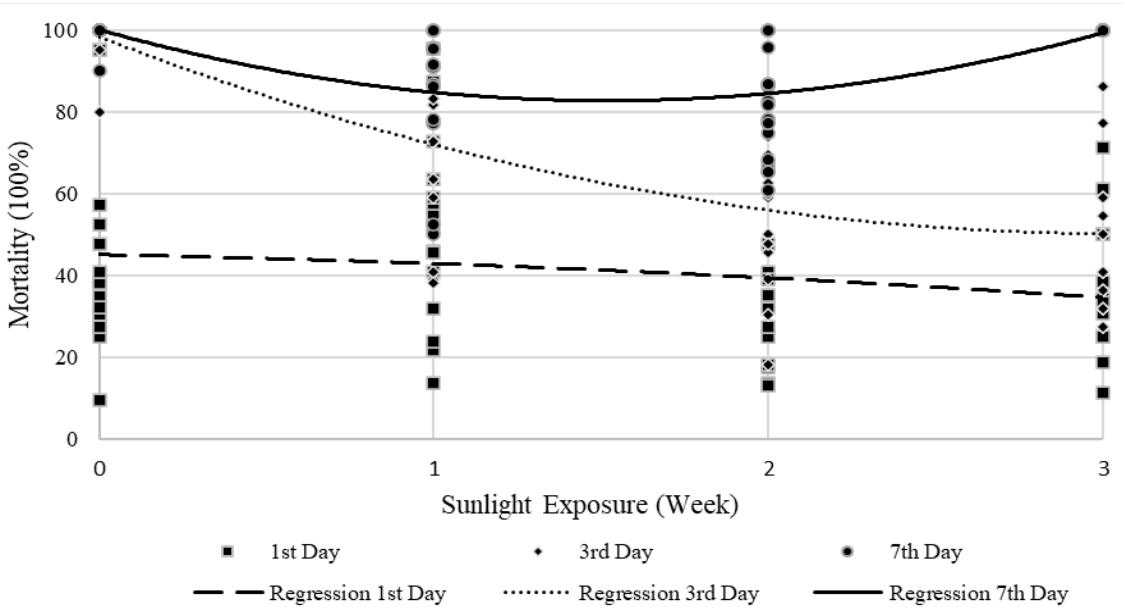

Figure 2. The interaction of *S. litura* mortality and sunlight exposure (week) at first, second, and third day after treatment of *B. thuringiensis*.

Table 3. Regression equation on different days of *S. litura* mortality after treated with *B. thuringiensis* at different mixtures.

Day of Mortality	R^2	Equation Regression (Quadratic Model)
1st Day	0.03	$y = -0.6611x^2 - 1.524x + 45.042$
3rd Day	0.58	$y = 5.1582x^2 - 31.52x + 98.357$
7th Day	0.39	$y = 7.5525x^2 - 22.908x + 100.13$

Note: y= mortality of *S. litura*; x = times of exposure (weeks).

0.58 on the third day. This finding suggested that the interaction pattern of sunlight exposure was as a quadratic model.

Cocoon extract of 2.5% *A. atlas* protected *B. thuringiensis* up to three weeks of sunlight exposure. The decreased of mortality was followed by the increased exposure time on the first, third, and seventh days after treatments showing quadratic model (Figure 2). The previous research reported that 2.5% of *A. atlas* cocoon extract protected *B. thuringiensis* up to the fourth week of exposure UV B (Sukirno et al. 2021). The cocoon of *A. atlas* containing sericin and protein fibroin (Fabiani et al. 1996) protected *B. thuringiensis* from sunlight. The mortality was decreased due to destruction of tryptophan and loss of biological activity (Cohen et al. 1991).

The comparison of *S. litura* mortality at the first day after treated with *B. thuringiensis* is showed in Table 4. The *S. litura* in *B. thuringiensis* formulations which were exposed to sunlight for two and three weeks, was no significant difference. However, at week-0 and week-1, the protection of *A. atlas* cocoon extract treatments and mortality showed significant difference in exposed *Bt.* with extract and unexposed *Bt.* with extract.

On the third day of mortality, the treatment of cocoon extract of *A. atlas* was not a significant difference. Nevertheless, the average mortality declined with the increasing time of exposure. The pathogenicity of *B. thuringiensis* against *S. litura* reached 100% at zero week, whereas, the lowest mortality was 32% at the third week of exposure. The *S. litura* mortality in the treatment of one week sunlight exposure was significant difference in exposed *Bt.* with extract, which was recorded on the 7th day.

Table 4. The average mortality (%) of first instar larvae of *S. litura* (mean ± SE) treated *B. thuringiensis* formulated of 2.5% *A. atlas* cocoon extract at different exposure under sunlight.

Treatments	Sunlight Exposure (Week)			
	0	1	2	3
Mortality of *S. litura* on the first day (%)				
Bt + extract + exposure	79.37 ± 15.87 Ab	27.03 ± 5.94 Aa	20.49 ± 7.15 Aa	23.94 ± 7.12 Aa
Bt - extract + exposure	41.24 ±7.95 Ba	51.02 ± 14.39 Aab	21.77 ± 4.41 Aa	51.59 ± 11.03 Aa
Bt + extract - exposure	34.33 ± 2.93 Ba	68.41 ± 9.75 Bb	38.89 ± 3.52 Aa	44.91 ± 6.13 Aab
Bt - extract - exposure	25.89 ± 5.67 Ba	50.51 ± 3.52 Aba	35.34 ± 11.59 Aa	24.84 ± 3.47 Aa
Mortality of *S. litura* on the third day (%)				
Bt + extract + exposure	100.00±0.00 Ab	50.69±7.72 Aa	35.05±8.80 Aa	39.39±5.46 ABa
Bt - extract + exposure	96.00±4.00 Ac	68.66±12.67 ABbc	47.10±6.62 ABab	32.95±3.41 Aa
Bt + extract - exposure	100.00±0.00 Ac	86.52±4.59 Bbc	67.97±3.65 Bab	54.55±7.33 ABab
Bt - extract - exposure	99.05±0.95 Ab	72.32±3.92 ABab	68.42±8.63 Bb	68.18±11.13Bb
Mortality of *S. litura* on the seventh day (%)				
Bt + extract + exposure	100.00 ± 0.00 Ab	91.03 ± 4.28 Aa	74.51 ± 6.42 Ab	100.00 ± 0.00 b
Bt - extract + exposure	98.00 ± 2.00 Aa	77.79 ± 10.87 Aa	88.75 ± 4.97 Aa	100.00 ± 0.00 a
Bt + extract - exposure	100.00 ± 0.00 Ab	89.24 ± 3.71 Aa	87.51 ± 3.62 Aa	100.00 ± 0.00 b
Bt - extract - exposure	100.00 ± 0.00 Ab	88.53 ± 4.59 Aab	79.80 ± 5.62 Aa	100.00 ± 0.00 b

Notes: The average mortality and SE within the same column followed by uppercase letters are not significantly different tested at $\alpha < 0.05$. The average mortality and SE within the same row followed by lowercase letters are not significantly different tested at $\alpha < 0.05$.

Furthermore, the mechanisms of action of *B. thuringiensis* were: (1) protoxin was solubilized to be activated in the midgut. Protoxin activated when dissolved in alkaline and highly acid insect midgut; (2) after activated, place toxin bound by receptors (specific protein) in the apical brush border membrane midgut; (3) toxin forms pores and losses cell development; (4) pores within the cell produce air, and other ions entered. The cells swell and eventually lyses. The cells were destroyed, lost growth, and caused death (Khetan 2001).

The SEM (Scanning Electron Microscope) analysis of *B. thuringiensis* formulation

The experiment was using a commercial *B. thuringiensis* DiPel-WP serotype *kurstaki* HD-7. DiPel-WP was commonly made with some ingredients that kept the quality of *B. thuringiensis* under natural conditions, rain, and exposure to sunlight (Ignoffo et al. 1977). Under natural conditions, *B. thuringiensis* commercially survived in 42-56% RH (Teera-Arunsiri et al. 2003). SEM was used to know the different surfaces of treatments. The *B. thuringiensis* which was formulated with the addition of *A. atlas* cocoon extract and *B. thuringiensis* alone were exposed under sunlight, then was observed using SEM with 3 different samples and with various magnifications.

Based on SEM, the surface of three samples is similar, because DiPel-WP was commonly made with some ingredients to be granule. So, that part of *B. thuringiensis* was not clearly visible in SEM. On SEM crystal and spores of unexposed *B. thuringiensis* from DiPel-WP suspension showed a coarse solid (Figure 3A), whereas *B. thuringiensis* alone showed smoother texture than the powder formulated on the surface (Teera-Arunsiri et al. 2003). DiPel suspension added of *A. atlas* cocoon extract with exposure to the sunlight for one week showed more rough surface (Figure 3B1.1) than *B. thuringiensis* alone. After being exposed for three

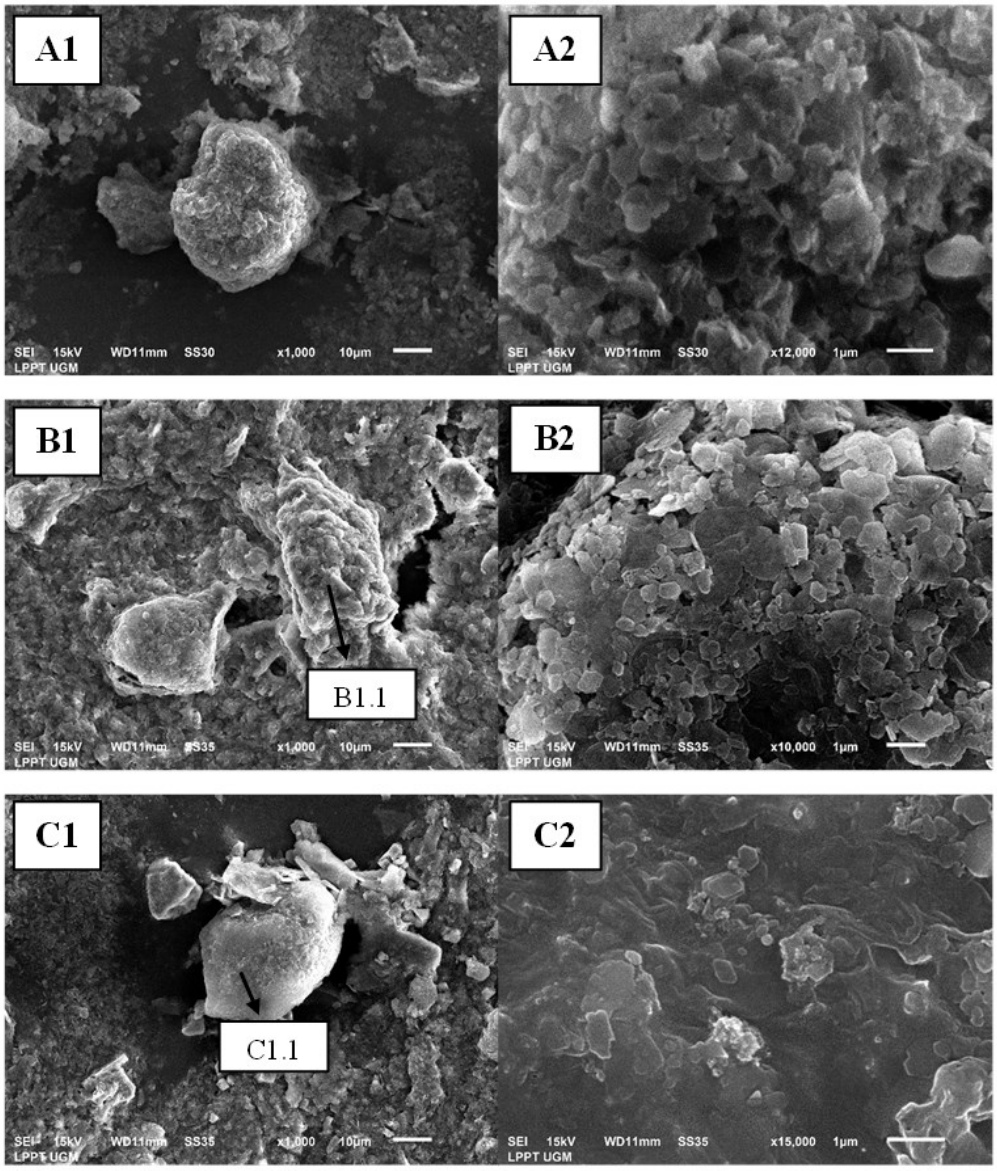

Figure 3. SEM analysis of *B. thuringiensis* formulations.
Note: (A1) Suspension of *B. thuringiensis* from DiPel-WP with 1,000 magnifications; (A2) Suspension of *B. thuringiensis* from DiPel-WP with 12,000 magnifications; (B1) Formulation of *B. thuringiensis* and 2.5% *A. atlas* cocoon extract exposed under the sunlight for one week with 1,000 magnifications; (B2) Formulation of *B. thuringiensis* and 2.5% *A. atlas* cocoon extract exposed under the sunlight for one week with 10,000 magnifications; (C1) Formulation of *B. thuringiensis* and 2.5% *A. atlas* cocoon extract exposed under the sunlight during three weeks with 1,000 magnifications; (C2) Formulation of *B. thuringiensis* and 2.5% *A. atlas* cocoon extract exposed under the sunlight during three weeks with 15,000 magnifications.

weeks, the particles showed a smooth surface (Figure 3C1; C2; C1.1). The addition of 2.5% *A. atlas* cocoon extract aimed to protect *B. thuringiensis* commercial from exposure to sunlight. Increased temperature to 100°C declined the survival of spores *B. thuringiensis* to 3% (Teera-Arunsiri et al. 2003).

CONCLUSION

This study showed that the mortality of *S. litura* decreases, followed by an increased time of exposure to sunlight. The result concluded that 2.5 % of *A. atlas* cocoon extract protects *B. thuringiensis* commercially for up to three weeks of exposure to sunlight. On the other hand, the mortality of *S. litura* was reaching maximum on the seven days after treatments. The suspension of *B. thuringiensis* and *A. atlas* cocoon extract presented differences in the surface at the other exposure times.

AUTHORS CONTRIBUTION

N.S.N. conceived, designed the experiments, wrote the manuscript, reared and collected *S. litura*. R.R. conceived, designed the experiments, reared and collected *S. litura*. S.S. planned, organized, supervised the experiments, made critical revisions, and approved the final version. A.S.P.W., N.S.S.S., H.A., A.A., T.P.S., and H.A. reared and collected *S. litura*.

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CONFLICT OF INTEREST

The authors state that there was no conflict of interest in this research.

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