UV Protectant Ability of *Attacus atlas* L. (Lepidoptera: Saturniidae) Sericin Extract to Increase Nucleopolyhedrovirus Effectiveness against Beet Army Worm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)

Hana Widiawati[1] Sukirno Sukirno[2] Siti Sumarmi[2] Hari Purwanto[2] R.C. Hidayat Soesilohadi[2] Ignatius Sudaryadi[2,*]

[1]*Tropical Biology Department, Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Selatan, Sekip Utara, Bulaksumur Yogyakarta 55281, Indonesia.*
[2]*Entomology Laboratory, Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Selatan, Sekip Utara, Bulaksumur Yogyakarta 55281, Indonesia.*
[*]*Corresponding author.Email : dsudaryadi@ugm.ac.id*

ABSTRACT
Spodoptera exigua (Lepidoptera: Noctuidae) is the common pest known for attacking shallot crop. Baculovirus (Nucleopolyhedrovirus : NPV) is a biological agent that is widely used as the pest control agent. However, the activity of NPV is deteriorated when applied in the field due to the influence of ultraviolet from the sun. *Attacus atlas* silkworm cocoon has UV protectant ability due to the presence of sericin protein. Thus, the aim of this study is to determine the degree of UV protectant ability from *A. atlas* cocoon extract for NPV to prevent its deterioration and to increase its effectiveness against *S. exigua* pest. The stages of this study consist of UVB irradiation of *A. atlas* cocoon extract as NPV protectant and pathogenicity test of NPV activity against the first instar larvae of *S. exigua*. Experiments were carried out by exposing NPV to UVB irradiation for 0, 1, 2, 3, 4 weeks long with the addition of *A. atlas* cocoon extract in varied concentrations (0, 0.5, 1, 2, and 2.5%). The concentrations derived from 7.5 gram *A. atlas* cocoon was dilluted 1 gram TRO and 150 ml aquades. The results proved the UV protectant ability of *A. atlas* cocoon for NPV. After a week of UVB irradiation, the NPV applied in *A. atlas* cocoon extract (0-2.5%) caused larval mortality of 43.33%, 76.67%, 60%, 65%, 80%, respectively. After 2 weeks of UVB irradiation, the NPV applied in *A. atlas* cocoon extract (0-2.5%) caused larval mortality of 46.67%, 78.33%, 71.67%, 66.67%, 66.67%, respectively. Thus, for further applications, the findings of this research need to be tested in an actual field condition.

Keywords : *Attacus atlas, Nucleopolyhedrovirus, Spodoptera exigua, Ultraviolet.*

1. INTRODUCTION

As an Agricultural country, Indonesia has many agricultural sectors including fruits and vegetables. One type of agricultural field widely developed in Indonesia is shallot crop. Shallot is used for spices and food flavorings. Shallot production is still unstable due to several constraints such as pest and diseases. The most common pest of shallot crop is beet army worm known as *Spodoptera exigua* (Lepidoptera : Noctuidae). *S. exigua* becomes pest at its larval stage. With a density of only 1.3 – 2.4 larvae per clump, *S. exigua* can cause serious destruction to the production of shallot [1]. The larvae consume the leaves which affect the plant growth and decrease the quality and quantity of the harvest [2]. Chemical insecticide is commonly used to overcome this problem. However, the use of chemical insecticide

has some negative impacts to the environment and human health. Recently, people's awareness towards environmental condition and safety in food production has been increasing and there has been an increased attention in using alternative methods to decrease beet army worm population [3]. Biological control approach using Baculovirus (Nucleopolyhedrovirus : NPV) is one of the solutions to control *S. exigua.* Previous research showed that Baculovirus can cause a larval mortality of 91.67% with a suspension of 30 larvae infected with the virus / 1 liter of water [4]. Baculovirus can also be used as agent to control noctuid moths pest, such as *Helicoverpa armigera* and *Spodoptera exigua* [5]. However, the pathogenicity of Nucleopolyhedrovirus can be inhibited by UV radiation from the sun [2]. Ultraviolet from the sunlight is the main factor that destroys microbial control agents. The activity of microbial control agents, including Baculovirus, can be inactivated by the ultraviolet B (280-320 nm wavelength) of sunlight [6,7]. Thus, in order to improve the pathogenicity of Baculovirus, some ultraviolet protectant from natural ingredients have been used as additives.

One natural ingredient that has the potential of UV protectant ability is *A. atlas* cocoon because it contains sericin protein which has anti-UV activity [8,9]. *A. atlas* cocoon contains 2 types of protein, fibroin protein and sericin protein. Fibroin protein makes up 70-80% of *A. atlas* silkworm cocoon layer while sericin protein makes up 20-30% of the cocoon layer of *A. atlas*. *A. atlas* cocoons contain several amino acids such as alanine, tyrosine, glycine, serine, aspartic acid, arginine, glutamic acid, and histidine [8]. Secirin protein from *A. atlas* cocoon is considered as a waste material [10]. Sericin is known as an anti-UV compound because it has antioxidants and anti-tyrosinase activity to ward off free radicals [11]. Research shows that sericin protein can suppress ultraviolet-B-induced and chemically-induced damage and tumor in mouse skin [12]. Therefore, silkworm cocoon extract has been widely used in the cosmetic industry [8]. *A. atlas* cocoon extract in 2.5% concentration has also been proven to protect *Bt* from UV damage [10]. Due to its sericin content, *A. atlas* extract is thus able to improve the pathogenicity of Baculovirus in controlling *S. exigua* pest.

The aim of this research is to know the effectiveness of *A. atlas* cocoon as ultraviolet protectant for Nucleopolyhedrovirus in order to increase its pathogenicity against the first instar larvae of *S. exigua*. In the future, this research is encouraged to be used as literature background for the continuing research and the effectivity results can also be used as reference for the future application of Nucleopolyhedrovirus protectant by farmer to decrease the number of *S. exigua* pest in the shallot crop.

2. MATERIALS AND METHODS

The main materials used in this research are the test insect or beet army worm (*Spodoptera exigua*), *Attacus atlas* silkworm cocoons, and the microbial agent *Spodoptera littoralis* Multiple Nocleo Polyhedro Virus (*Spli*MNPV).

2.1 Making the artificial diet

The test insects were maintained in the Entomology Laboratory on a artificial diet made from white beans [13]. White beans (250 g) were submerged in water for overnight and were cooked afterwards. The cooked beans were blended with 1200 ml aquades and were added with 80 g of yeast, 50 g of agar, and 10 g of benzoic acid. Those materials were boiled and were then added with 20 g of ascorbic acid.

2.2 Test Insect Sampling and Rearing

The beet armyworm *S. exigua* were collected directly from the welsh onion crop in Paten, Dukun, Magelang. Test insects were maintained in the Entomology Laboratory, Faculty of Biology, Universitas Gadjah Mada. The larvae of *S. exigua* were maintained on an artificial diet made from white beans. The imago of *S. exigua* were maintained with honey.

2.3 Extraction of Attacus atlas Cocoon Sericin

The extract was obtained using degumming method to separate fibroin and sericin. Cocoon weighted 7.5 g were mixed with 50 ml aquades and 1 g TRO (Turkey Reddish Oil). The materials were boiled in 350º C for an hour. The solution obtained was stored for 24 hours. The solution obtained (20 ml) was centrifuged at 4º C, 4000 rpm for 10 minutes. The extract had a concentration of 5% (sample A). The cocoon of *A. atlas* was also extracted using mechanical method. *A. atlas* cocoon weighted 100 mg was mixed with 50 µl sample buffer nad 50 µl PBS 10x (sample B).

2.4 Measuring the Molecular Weight of Sericin Protein from Attacus atlas cocoon

The molecular weight of sericin protein was measured using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Separating gel (12%) and Stacking gel (12%) were used. 5 ml of sample A was precipitated with 5 ml of aceton (ratio 1 : 1) and stored overnight. The solution was centrifuged in 4000 rpm for 5 minutes until sediments appeared. The aceton was removed and stored in the oven for 50º C until dry. The sample was added with 100 µl PBS 10x and 100 µl sample buffer. The sample was then added with 2 µl betamercaptoethanol. The mixture was put into water

bath for 5 minutes (sample A). Mechanically extracted 100 mg *A. atlas* cocoon was mixed with 50 µl PBS 10x and 50 µl sample buffer. The sample was then added 2 µl betamercaptoethanol and vortexed (sample B).

Each sample of cocoon extract (8 µl) was added with 200 µl Bradford reagent and put into 96 wall plates. The absorbance of the sample was measured using Elisa reader at λ 595 nm to determine the volume for the comb. Twenty µl of sample A and 10.5 µl sample B were added to the comb and was run using Biorad Instalation on 50 volts for 50 minutes until it passed the stacking gel. The voltage was increased to 100 volts for 60 minutes to pass the separating gel. The gel was stained with Commasive Brilliant Blue for 24 hours. The gel was then destained using pure distilled water and oven every 2 minutes. The gel was then photographed using Godox and cellphone.

2.5 Absorbance measurement of Attacus atlas cocoon

The sample that was used for this step was sample A. The absorbance of the *A. atlas* cocoon extract was determined using Spectrophotometer UV-Visible. The 5% concentration of *A. atlas* extract was dilluted to 2.5, 2, 1, and 0.5%. The absorbance of each concentration was measured by using 10 ml of each sample and was exposed under UV-B radiation at 190-420 nm wavelength.

2.6 Preparing Nucleopolyhedrovirus and Ultraviolet-B Exposure Under Laboratory Conditions

The virus used was *Spodoptera littoralis* Multiple Nocleo Polyhedro Virus (*Spli*MNPV). *Spli*MNPV has the commercial product named Littovir (Biocontrol, Switzerland). The concentration of Littovir was $2x10^9$ PIB/ml (Polyhedral Inclusion Body) [3]. Each NPV was added with aquades (control), 0.5, 1, 2, 2.5% *A. atlas* cocoon extract. The samples were then exposured under UV-B radiation for 0, 1, 2, 3, and 4 weeks. Each sample was repeated 3 times. Each sample was added with 10 ml of aquades to get the concentration of NPV at Lc95 (2×10^8 PIB/ml). The samples were stored at 4º C.

2.7 Counting the Number of Nucleopolyhedrovirus After UV-B Exposure

The number of NPV was counted using counting chamber method [14]. NPV (8.5 µl) was put on the haemocytometer. The number of NPV in 1 mm^3 can be calculated using Equation (1), where *D* is dilution factor, *X* is NPV counted, *N* is the number of squares counted, and *K* is volume of the smallest square (cm^3).

$$\text{The number of NPV} = \frac{D \times X}{N \times K} \quad (1)[14].$$

2.8 Pathogenicity Test of Nucleopolyhedrovirus Against Spodoptera exigua Larvae

Pathogenicity test was done with combinations of concentration treatments (0, 0.5, 1, 2, and 2.5%) and UV-B duration treatments (0, 1, 2, 3, and 4 weeks). Each combination was repeated 3 times. NPV (500 µl) was put on the artificial diet and 20 *S. exigua* first instar larvae were put on the artificial diet as well. The mortality of the *S. exigua* was examined for 7 days and was calculated using Equation (2), where *x* is the number of dead larvae, *k* is the number of initial larvae.

$$\text{Mortality} = \frac{x}{k} \times 100\% \quad (2)[15].$$

2.9 Statistical Analysis

Statistical analysis was done using SPSS 13. The means of the number of NPV and means of the mortality *S. exigua* larvae were used for the analysis of variance (ANOVA) to test the differences among the treatments. Advanced analysis was done using Tukey HSD to determine the differences.

3. RESULT AND DISCUSSION

The protein of *A. atlas* silkworm cocoon is showed in figure 1. Determination of protein molecular weight from *A. atlas* cocoon extract was carried out using the Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) method. SDS-PAGE can be used to identify protein types based on their protein

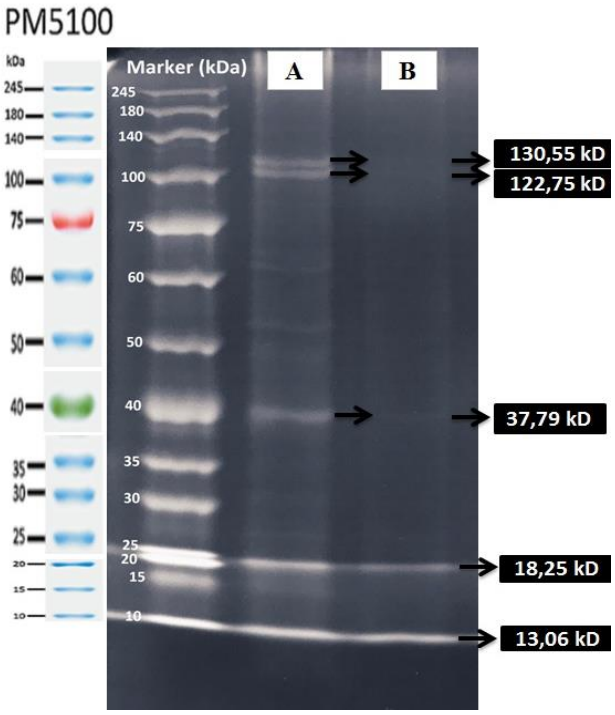

Figure 1. Sericin protein from *Attacus atlas* cocoon extract, TRO (A) and mechanical extraction (B).

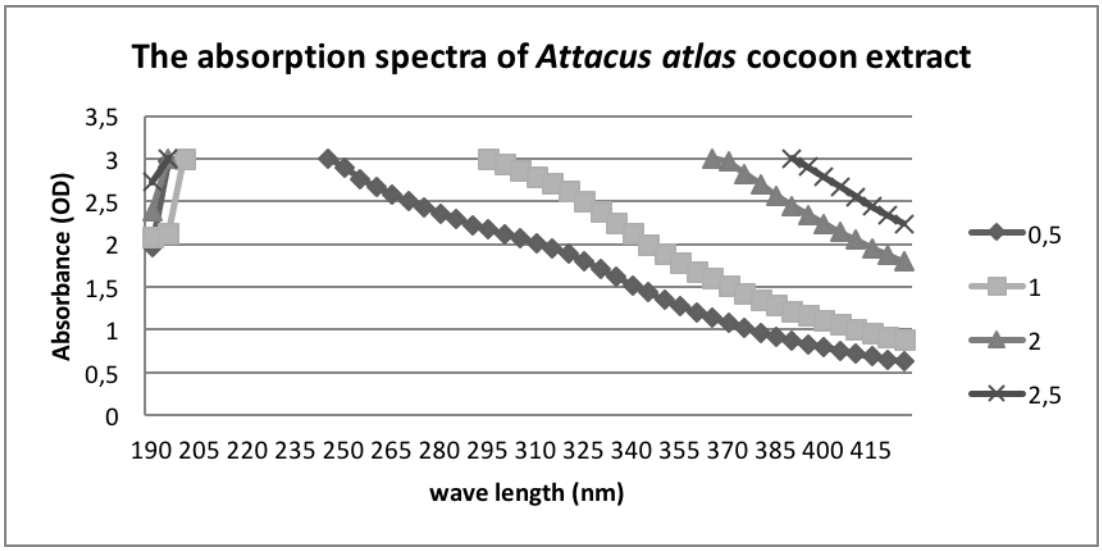

Figure 2. The absorption spectra of *Attacus atlas* cocoon extract 0, 0.5, 1, 2, 2.5 % measured by spectrophotometer at the wavelength range of 190-420 nm.

molecular weight. The protein contained in the cocoon extract of *A. atlas* was indicated by the formation of bands on the electrophoresis gel. The results in the form of these bands were used in linear regression equations in order to calculate the molecular weight of each protein [16].

Based on Figure 1, there were more bands of protein observed from TRO (Turkey Reddish Oil) extraction compared to extraction using mechanical method. Sericin protein consists of 67% hidrofobic amino acids, so mechanical extraction cannot separate sericin from fibroin protein. TRO contains detergent which can degrade cell membrane, thus the TRO can separate sericin from the fibroin protein [8]. There were 5 bands of protein extracted using TRO with molecular weight of 130.55, 122.75, 37.79, 18.25, and 13.06 kDa. There were 2 bands of protein extracted using mechanical method with molecular weight of 18.25 and 13.06 kDa. Sericin protein has molecular weight ranging between 40-176 kDa [17]. Sericin protein also has molecular weight ranging between 20-310 kDa [18]. We can conclude that *A. atlas* silkworm cocoon extract obtained for this research contains sericin protein. Sericin protein from *A. atlas* cocoon has the function to protect the pupae from ultraviolet radiation [11].

The wavelength used for the spectrophotometry analysis for this research ranges from 190 - 420 nm because it shows the wavelength of ultraviolet spectrum. UV radiation consists of UVA (320-400 nm), UVB (280-320), and UVC (200-280) [6]. This research showed that all of the *A. atlas* cocoon extract concentration variations (0.5, 1, 2, and 2.5%) can be read in the wavelength (λ) of 190 - 420 nm. Higher concentration of *A. atlas* cocoon extract resulted in higher absorbance value. This result was in accordance with the Lamber Bert Law which stated that UV radiation absorbed by a liquid is comparable with the concentration of the substance in the liquid. Based on this research, 0.5% *A. atlas* cocoon extract was seen from λ 190 nm and had the highest absorbance of 3.0 OD at λ 200 to 245 nm. Thus this concentration can be used for UVB protection because it can absorb at λ 280 - 320 nm (UVB spectrum) with a relatively high absorbance value of 1.88 - 2.35 OD. If arranged from highest to lowest, the capabilities to absorb UV for the tested concentrations was 0.5<1< 2< 2.5% [10]. Using only 0.5% of *A. atlas* cocoon extract as UV-B protectant will also reduce the cost of pest control.

To determine the degree of UV protectant ability from *A. atlas* cocoon extract, further test was carried out in the form of the NPV test under UV radiation and NPV pathogenicity test for *S. exigua* first instar larvae. The UV rays used against the NPV was UV-B radiation. UV-B radiation is a solar energy that influence the biological activity of living things by causing the formation of free radicals in living things [19].

The number of Nucleopolyhedroviruses decreases with the increasing duration of UV exposure. The number of NPV without the addition *A. atlas* cocoon extract was fewer than the number of NPV with added *A. atlas* cocoon extract. Without the addition of *A. atlas* cocoon extract, the NPVs will be destroyed by UVB rays. UV rays can cause inactivation by forming cross links between nucleic acid in the form of dimer pirimidin (timin) formation. This causes damage to the genetic material of Nucleopolyhedrovirus. Nucleopolyhedrovirus genetic material has the form of double-stranded DNA. The DNA contains pyrimidine nitrogen bases (thymine and cytosine) and purine nitrogen bases (guanine and adenine). Absorption of ultraviolet light can lead to the formation of pyrimidine dimers in the DNA strand which can cause mutation and

Table 1. The number of NPV ± SE (x10^6 PIB/ ml) with added *A. atlas* cocoon extract concentrations 0, 0.5, 1, 2, 2.5% and UV-B exposure 0, 1, 2, 3, and 4 weeks.

Treatments	UV B Exposure (Week)				
	0	1	2	3	4
NPV + H_2O	198.92 ±1.08 ^{Aa}	156.50± 24.63 ^{Aa}	39.3 ±12.44 ^{Ba}	26.25± 11.1 ^{Ba}	16.08 ± 6.80 ^{Ba}
NPV + 0.5%	186.42 ± 9.15 ^{Aa}	174.17 ± 19.37 ^{Aa}	69.25 ± 15.97 ^{Ba}	44.50 ± 15.46 ^{Bab}	16.08 ± 4.95 ^{Ba}
NPV + 1%	193.67 ± 3.76 ^{Aa}	162.33± 6.51 ^{Aa}	74.58 ± 35.48 ^{Ba}	79.00 ± 5.5 ^{Bb}	17.50 ± 2.57 ^{Ba}
NPV + 2%	184.42 ± 8.20 ^{Aa}	193.25± 2.38 ^{Aa}	99.00± 21.88 ^{Ba}	78.25 ± 8.12 ^{BCb}	39.00 ± 9.26 ^{Ca}
NPV + 2.5%	204.75 ± 15.46 ^{Aa}	175.83± 6.80 ^{ABa}	114.33 ± 19.98 ^{BCa}	49.92 ± 1.75 ^{Cab}	68.42 ± 2.50 ^{Ca}

Means with different capital letter in same concentration of *A. atlas* cocoon extract are significantly different at α= 0.05. Means with different lower letter in same UV-B exposure are significantly different at α= 0.05.

death. The primary dimer formed in DNA due to UV exposure is thymine dimers [6,20]. UV-B radiation is one of the most important solar energy that can cause the formation of 3 types of DNA damages namely cyclobutane pyrimidine dimer (CPD), photoproduct pyrimidine 6-4 pyrimidine (6-4 PPs), and Dewar isomer [21].

Based on the One Way ANOVA analysis, different concentrations of A. atlas cocoon extract gave significant result for the number of NPV at α = 0.05 The results showed that after UV-B radiation for 1 week, A. atlas cocoon extract with an optimal 2% concentration maintained 193.25 ± 2.38x106 PIB / ml. After 2 weeks of UV-B radiation, the optimal 2.5% concentration of A. atlas cocoon extract maintained 114.33 ± 19.98x106 PIB / ml. Furthermore, after 3 weeks of UV-B radiation, the optimal 1% concentration of A. atlas cocoon extract maintained 79.00 ± 5.53x106 PIB / ml. After 4 weeks, the 2.5% extract maintained 68.42 ± 2.50x106 PIB ml. These data suggested that Nucleopolyhedrovirus is

Table 2. Mortality ± SE (%) *S. exigua* larvae with NPV added *A. atlas* cocoon extract concentrations 0, 0.5, 1, 2, 2.5% and UV-B exposure 0, 1, 2, 3, and 4 weeks.

Treatments	UV B Exposure (week)				
	0	1	2	3	4
NPV+H_2O	100 ±0 ^{Aa}	43.33 ± 23.51 ^{Aa}	46.67 ± 3.33 ^{Aa}	40.00 ± 22.55 ^{Aa}	41.67 ± 14.24 ^{Aa}
NPV+Extract 0.5%	100 ±0 ^{Aa}	76.67 ± 11.67 ^{ABa}	78.33 ± 11.67 ^{ABa}	55.00 ± 17.32 ^{ABa}	45.00 ±8.66 ^{Ba}
NPV+Extract 1%	100 ±0 ^{Aa}	60.00 ± 17.32 ^{Aa}	71.67 ± 11.67 ^{Aa}	70.00 ± 16.07 ^{Aa}	65.00 ± 11.55 ^{Aa}
NPV+Extract 2%	100 ±0 ^{Aa}	65.00 ± 7.64 ^{Aa}	66.67 ± 15.90 ^{Aa}	71.67 ± 13.33 ^{Aa}	61.67 ± 6.01 ^{Aa}
NPV+Extract 2.5%	100 ±0 ^{Aa}	80.00 ± 5.00 ^{ABa}	66.67 ± 16.41 ^{ABa}	65.00 ±12.58 ^{ABa}	48.33 ± 10.14^{Ba}

Means with different capital letter in same concentration extract are significantly different at α= 0.05.
Means with different lower letter in same UV-B exposure are significantly different at α= 0.05.

pathogenic to the first instar larave of S. exigua. The Table 2. shows the mortality of S. exigua first instar larvae treated without cocoon extract of A. atlas and with the addition of A. atlas cocoon extract for 0 week to 4 weeks. Based on the ANOVA analysis, the results showed that between the treatments (0%; 0.5%; 1%; 2%; 2.5%) there was insignificant result at the level of α = 0.05. In the treatment without the addition of A. atlas cocoon extract, the mortality of S. exigua larvae had a high standard error. After a week of UV-B radiation it caused a mortality of 43.33 ± 23.51% and after 3 weeks of UV-B radiation it caused a mortality of 40.00 ± 22.55%. The high standard error was due to the suboptimal performance of NPV as a biological agent without a protectant that protects the NPV from UV-B radiation. The pathogenicity of NPV against S. exigua larvae is inhibited by the damage to NPV particles caused by UV-B radiation.

In the treatment with the addition of *A. atlas* cocoon extract, the mortality of *S. exigua* larvae had a relatively low standard error. The standard error of treatment with protectants did not reach half of the mortality data. This is because the addition of *A. atlas* cocoon extract is able to protect NPV from UV-B radiation, so NPV performance remains undisturbed and thus remains pathogenic to *S. exigua* larvae. After 1 and 2 weeks of UV-B radiation, the mortality of larvae without protection was 43.33 ± 23.51% and 46.67 ± 3.33%, respectively. On the other hand, the treatment with *A. atlas* cocoon extract protectant with a concentration of 0.5%, after 1 and 2 weeks of UV-B radiation, the mortality of *S. exigua* larvae was 76.67 ± 11.67% and 78.33 ± 11.67%, respectively. This showed that the mortality of *S. exigua* larvae is 1.5 to 2 times higher than in the control treatment. This is in accordance with the research that stated that sericin protein has antioxidant activity [22]. Antioxidants in sericin protein can prevent the formation of pyrimidine dimers, so the formation of free radicals that cause genetic damage to NPV can be prevented so that the NPV pathogenicity performance remains optimal against *S. exigua* larvae. The anti-ultraviolet activity of *A. atlas* cocoon extract is caused by the presence of phenol and flavonoid compounds in the cocoon layer [23]. *A. atlas* silkworms that are fed keben leaves or *Barringtonia asiatica* will produce cocoons containing secondary metabolites such as alkaloids, tannins, and flavonoids [24].

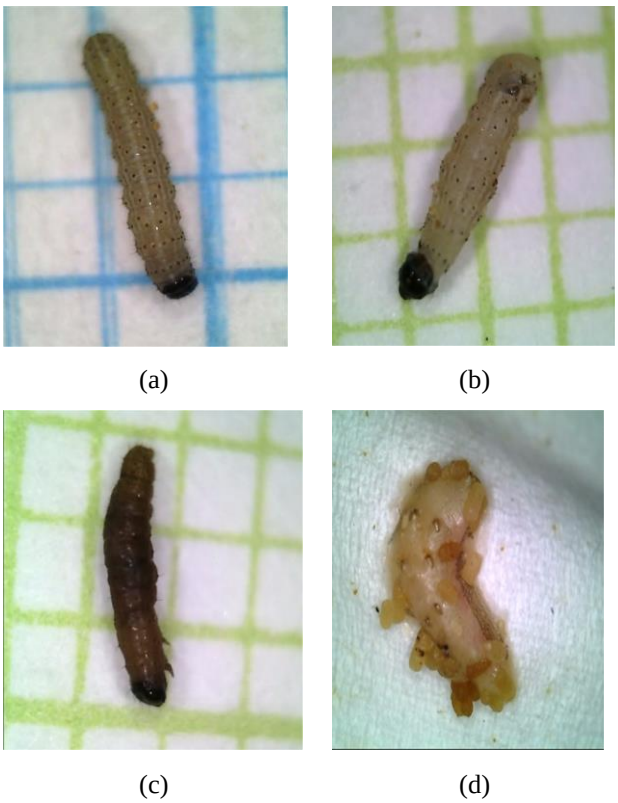

Figure 3. Morphological of *Spodoptera exigua* larvae, healthy larvae (a) and NPV-infected larvae (b,c,d).

Based on the mortality results of *S. exigua* larvae, it can be concluded that the concentration of *A. atlas* cocoon extract of 0.5% resulted in 1.5-2 times higher mortality of *S. exigua* larvae compared to treatment without *A. atlas* cocoon extract. After UV-B radiation for 1 and 2 weeks, *S. exigua* larvae experienced a mortality of 76.67 and 78.33%. Therefore, a concentration of 0.5% is optimal enough to be used as UV-B protectant because it can maintain the pathogenicity of NPV and can cause mortality in *S. exigua* larvae effectively. Previous research proved the UV protectant ability from *A. atlas* extract (2.5%) used for Bt can cause the mortality of *S. litura* 98.33% after 4 weeks UV B exposure [10]. The other sericin protein from *Samia ricini* 0.5% cause the *S. litura*'s mortality 86.67% after 4 weeks of UV B exposure [10].

S. exigua larvae which had been infected with NPV experienced color change gradually. According to this research, the gradual color change occured after 3 days of being infected by NPV. The larvae looked brighter and glossier with a bulging abdomen (Figure 3b). After day 4 of the infection, the larvae's color turned darker (Figure 3c). On day 6 after infection, the larval integument became fragile and looked like it was filled with fluid and thus became more swollen. *S. exigua* larvae infected with NPV became fragile and could easily be destroyed when touched because their bodies contained fluid in the form of virus particles. The cuticle of *S. exigua* larvae also became more fragile due to the activity of the cathepsin and chitinase genes contained in the Baculovirus genome. This gene has the function to damage the peritrophic matrix in the digestive tract of *S. exigua* larvae [25].

Based on the results of this research, it can be concluded that silkworm cocoon extract of *A. atlas* is able to protect NPV from damage caused by UV-B radiation and the addition of 0.5% *A. atlas* cocoon extract was effective enough to increase the

pathogenicity of NPV in order to control *S. exigua* pest. For the next research, findings of this research should be applied in the field trial condition.

AUTHORS' CONTRIBUTION

All authors have same contribution for this research and publication. S.Sk., S.Sm., H.P., R.C., and I.S. designed the research and supervised all the process, H.W. collected the data and analyzed the data. H.W., S.Sk. and I.S. wrote the manuscript.

ACKNOWLEDGMENTS

Thanks are conveyed to the RTA-UGM 2021 Grant for the financial support and our gratitude were delivered to The Faculty of Biology UGM and The PDUPT-Entomology laboratory research team for the facilities

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest from this manuscript.

REFERENCES

[1] D. Marsadi, I.W. Supartha, A.A.A.A.S. Sunari, Invasi dan tingkat serangan ulat bawang (*Spodoptera exigua* Hubner) pada dua kultivar tanaman bawang merah di Desa Songan, Kecamatan Kintamani, Kabupaten Bangli [Invasion and attack level of Beet Armyworm (*Spodoptera exigua* Hubner) on two cultivars of onio, E-Jurnal Agroekoteknologi Tropika. vol. 6(4), 2017, pp. 361–9.

[2] S. Sukirno, M. Tufail, K.G. Rasool, S. Salamouny, K.D. Sutanto, S.A. Aldawood, The efficacy and persistence of *Spodoptera littoralis* Nucleopolyhedrovirus (SpliMNPV) applied in UV protectants against the beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) under Saudi Field Conditions, Pakistan Journal of Zoology, vol. 50(5), 2015, pp. 1895–902.

[3] K.D. Sutanto, S. Salamouny, M. Tufail, K.G. Rasool, S. Sukirno, M. Shepard, *et al.*, Evaluation of natural additives to enhance the persistence of *Spodoptera littoralis* (Lepidoptera : Noctuidae) Nucleopolyhedrovirus (Spli MNPV) under field conditions in Saudi Arabia, Journal of Economic Entomology, vol. 0(0), 2017, pp. 1–7.

[4] A.S. Rimadhani, D. Bakti, M.C. Tobing, Virulensi nuclear polyhedrosis virus (NPV) terhadap ulat grayak (*Spodoptera litura* F.) (Lepidoptera : Noctuidae) pada tanaman tembakau Deli di rumah kaca [Nuclear polyhedrosis virus (npv) virulation against beet armyworm (*Spodoptera litura* F.) (Lepidopter), Jurnal Online Agroekoteknolog, vol. 1(3), 2013, pp. 678–88.

[5] L.M. de Souza, B.M.E. de Castro, W. Sihler, E. Krol, S. Boguslaw, Baculoviruses: A Safe Alternative in Pest Control? Pest Technology, vol. 1(1), 2007, pp. 53–60.

[6] J. Kowalski, Ultraviolet Germicidal Irradiation Handbook, Berlin, SPRINGER, 2011, pp. 20–21.

[7] EPA, Glossary for UV Database. Office of Research and Development/National Exposure Research Laboratory/Human Exposure and Atmospheric Sciences Division, [Internet], 2007. Available from: https://ofmpub.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details=&vocabName=Glossary for UV Database.

[8] N. Reddy, Y. Zhao, Y. Yang, Structure and properties of cocoons and silk fibers produced by *Attacus atlas*, Journal of Polymers and the Environment, vol. 21(1), 2013, pp. 16–23.

[9] V.R. Silva, M. Ribani, M.L. Gimenes, A.P. Scheer, High molecular weight sericin obtained by high temperature and ultrafiltration process, In: Procedia Engineering [Internet], 2012, pp. 833–41. DOI: http://dx.doi.org/10.1016/j.proeng.2012.07.476

[10] S. Sukirno, D. Lukmawati, S.S.L. Hanum, V.F. Ameliya, S. Sumarmi, H. Purwanto, *et al.*, The effectiveness of *Samia ricini* Drury (Lepidoptera: Saturniidae) and *Attacus atlas* L. (Lepidoptera: Saturniidae) cocoon extracts as ultraviolet protectants of *Bacillus thuringiensis* for controlling *Spodoptera litura* Fab. (Lepidoptera: Noctuidae), International Journal of Tropical Insect Science. 2021.

[11] J. Kumar, B. Mandal, The inhibitory effect of silk sericin against ultraviolet-induced melanogenesis and its potential use in cosmeceutics as an anti-hyperpigmentation compound, [internet]. Available from:https://pubs.rsc.org/en/content/articlelanding/2019/pp/c9pp00059c#!divAbstract

[12] J.P. Kumar, S. Alam, A.K. Jain, K.M. Ansari, B.B. Mandal, Protective activity of silk sericin against UV radiation- induced skin damage by downregulating oxidative stress, ACS Applied Bio Materials, vol. 1(1), 2018, pp. 1–51.

[13] H. Shorey, R.L. Hale, Mass-Rearing of the larvae of nine Noctuid species on a simple artificial medium, Journal of Economic Entomology, vol. 58(3), 1965, pp. 522–4.

[14] D. Grzywacz, R. Rabindra, M. Brown, K. Jones, M. Parnell, The *Helicoverpa armigera* NPV Production Manual, FAO, 2004, pp. 71–76.

[15] D. Arlita, T. Hadiastono, M. Martosudiro, Pengaruh suhu awal terhadap infektivitas *Spodoptera litura* Nuclear Polyhedrosis Virus (SlNPV) JTM 97C untuk mengendalikan *Crocidolomia binotalis* zell. (Lepidoptera : Pyralidae) pada tanaman kubis (Brassica oleracea var. capitata L.) Effect of initial tem, Jurnal HPT, vol. 2(3), 2014, pp. 28–35.

[16] D. Holme, P. Hazel, Analytical biochemistry. England, 1998.

[17] G. Çapar, S. Aygun, Characterization of sericin protein recovered from silk wastewaters, Türk Hijyen ve Deneysel Biyoloji Dergisi, vol. 72(3), 2015, pp. 219–34.

[18] W. Tao, M. Li, R. Xie, Preparation and Structure of Porous Silk Sericin Materials, Macromolucular Materials and Engineering, vol. 290, 2005, pp. 188–94.

[19] S. Martin, J. Domek, Relative effects of ultraviolet and visible light on the activities of corn earworm and beet armyworm (Lepidoptera: Noctuidae) nucleopolyhedroviruses, Journal of economic entomology, vol. 95(2), 2002, pp. 261–8.

[20] M. Dizdagorlu, P. Jaruga, Mechanisms of free radical-induced damage to DNA. Free Radical Research, vol. 46(4), 2002, pp. 382–419.

[21] R.P. Rastogi, Richa, A. Kumar, M.B. Tyagi, R.P. Sinha, Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair, Journal of Nucleic Acids, vol. 2010(1), 2010, pp. 1–32.

[22] A. Rangi, L. Jajpura, The Biopolymer Sericin : Extraction and Applications, Textile Science & Engineering, vol. 5(1), 2015, pp. 1–5.

[23] G.A. Miguel, C. Álvarez-lópez, Extraction and antioxidant activity of sericin , a protein from silk, Brazilian Journal of Food Technology, vol. 23(1), 2020, pp. 1–14.

[24] L. Hidayati, T.R. Nuringtyas, Secondary metabolite profiling of four host plants leaves of wild silk moth *Attacus atlas* L, Indonesian Journal of Biotechnology, vol. 21(2), 2016, pp. 117–24.

[25] S. Samsudin, T. Santoso, Uji patologi Spodoptera exigua Nucleopolyhedrovirus (Se NPV) pada larva *Spodoptera exigua* Huebner (Lepidoptera : Noctuidae) [Pathological assay of *Spodoptera exigua* Nucleopolyhedrovirus (Se NPV) on the *Spodoptera exigu*a Huebner (Lepidoptera : Noctuidae) , Jurnal Biologi Indonesia, vol. 10(2), 2014, pp. 169–78.