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The Effects of Ultraviolet B on The Efficacy of *Bacillus thuringiensis* var. *kurstaki* Formulations Against Tobacco Armyworm, *Spodoptera litura* (Lepidoptera: Noctuidae)

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ABSTRACT

Tobacco armyworm (*Spodoptera litura* Fab.) is one of the major insect pests of crops in Indonesia. The management of this pest still depends on the use of chemical insecticides. The use of bio-insecticides, such as *Bacillus thuringiensis* (Bt.), are known to be alternatives, but it easily degraded by sunlight. This research aimed to study the effects of UV B on Bt. pathogenicity and to explore plant-based additives as UV B protectants for Bt. against armyworm. Thirteen plant extracts were screened based on their UV spectra absorbencies using UV spectrophotometry. The extracts, namely cloves, Jicama, Celebes pepper, turmeric, and Moringa, then used for the formulations of Bt. and exposed under UV B lights for 0, 72, and 144 h. After exposure to UV B, Bt. formulations were tested for bioassay against one- day-old 1st and one day old 2nd larval instars of tobacco armyworm. The result indicated that at 72 and 144 h of UV B exposures, the Bt. added formulations were signifcantly different compared to Bt. alone. At 72 h exposure against the 2nd larval instar, the larval mortality of tobacco armyworm on the second day of observation on Bt. formulated with Celebes pepper and turmeric was 97.3 and 80%, respectively, whereas, at 144 h exposure, the mortality was 96 and 89.3%, respectively. This study concluded that Celebes pepper and turmeric extracts were the potential to be used as the Bt. protectants against UV B.

1. Introduction

Tobacco armyworm, *Spodoptera litura* Fab. (Lepidoptera: Noctuidae), is a polyphagous insect pest of crops in Indonesia. Over 120 host plants, including crops, ornamental plants, weeds, wild plants, and trees, were recorded in 56 countries, including Indonesia (CABI 2018). In Indonesia, it was found in 22 provinces, and annually as much as 11,163 ha of crops were significantly affected (BALITKABI 2015). In soybeans, this insect caused 80% losses. In addition to soybeans, other host plants that armyworms can attack are chili, cabbage, rice, corn, tomatoes, sugar cane, beans, oranges, tobacco, shallots, eggplant, potatoes, beans, kale, spinach, banana, and ornamental plants as well as weeds *Limnocharis* sp., *Passiflora foetida* L., *Ageratum* sp., *Cleome* sp., *Clibadium* sp., and *Trema* sp. (BPPTP 2015).

Farmers depend on chemical insecticides for the pest management of the armyworm due to their quick killing potential. Unfortunately, its price made it difficult for the farmers to purchase the insecticides. On the other hand, the excessive use of insecticides is also known to endanger the environment and lead to pest resistance and human health. The residues of the insecticides also caused environmental pollution. Because of these, many countries have reduced the use of chemical insecticides in the agricultural sector, but in Indonesia alone, the use of chemical insecticides is still relatively high. The Indonesian government provides more than US$ 100 million in subsidies annually (Gallagher *et al.* 1994). Although a few decades ago, it was completely stopped, in the case of soybean production, it was estimated that

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more than 2 tons of insecticides were applied yearly (Mariyono 2008).

Biological agents that have been widely used for the biological control of insect pests is an entomopathogenic bacterium *Bacillus thuringiensis* (*Bt*.). This bacterium is safe, environmentally friendly, and specific against target pests. It is also renewable in nature. Thus, it is inexpensive and practically can be propagated by the farmers. Unfortunately, this bacterium is sensitive and easily degraded by climatic factors (Abbaszadeh *et al.* 2011; Moustafa *et al.* 2018; Van Bokhorst-van de Veen *et al.* 2015), for example, by ultraviolet sunlight. Ultraviolet is the main cause of cry toxin degradations (Pan *et al.* 2017). The ultraviolet B (UVB), which has 290 to 320 nm spectra, is the main factor for the *Bt.* inactivation (Myasnik *et al.* 2001; Zogo *et al.* 2019) by reducing the spore viability and the toxin integrity (De Oliveira *et al.* 2021; do Nascimento *et al.* 2022).

The persistence of *Bt.* in the field can be enhanced by using some materials, for example, Congo red and folic acid (Dunkle and Sasha 1989), protein and cosmetic product (Griego and Spence 1978), sepiolite (Zhou *et al.* 2018), silica nanoparticles (Wu *et al.* 2018), graphene oxide and olive oil (Maghsoudi and Jalali 2017), and melanin (Sansinenea and Ortiz 2015). Indigo carmine (Shapiro and Robertson 1990), carbon (Ignoffo *et al.* 1991), lignin (Elnagar *et al.* 2003; El Salamouny and Huber 2004; Tamez-Guerra *et al.* 2000), black tea, green tea, cocoa, and coffee (El Salamouny *et al.* 2009a, 2009b; Shapiro *et al.* 2008), spices extracts (Shapiro *et al.* 2009b; Shepard *et al.* 2010) have the potential as UV protectants for baculoviruses. Some plant extracts also had been reported to potentially improve the persistence of baculovirus, spinosyn, and neem when applied under arid conditions (Sutanto *et al.* 2017; Sukirno *et al.*, 2017, 2018).

Indonesia has high intensity of sunlight for the whole year; thus, it made the use of *Bt.* vulnerable to UV sunlight which degrades the pathogenicity and persistence. On the other hand, there are many indigenous plant materials which are the potential to be used as UV protectants for *Bt*. This study examined the effectiveness of plant extracts, namely jicama tuber (*Pachyrhizus erosus* (L.) Urb.), the rhizome of mango ginger (*Curcuma mangga* Val.), turmeric rhizome (*Curcuma longa* Linn.), cloves flower (*Syzygium aromaticum* (L.) Merrill and Perry), "Malang" green apple whole fruit (*Malus sylvestris* Mill.), betel leave (*Piper betel* L.), Celebes pepper leave (*Piper ornatum* N.E.Br.), green cabbage leave (*Brassica oleracea* L.), white cauliflower (*Brassica oleracea* L.), green cauliflower (*Brassica oleracea* L.), red cauliflower (*Brassica oleracea* L.), carrot tuber (*Daucus carota* (Hoffm.) Schubl and G. Martens), and Moringa leave (*Moringa oleifera* L.). in counteracting the UV B lights. Thus, the present study focuses on evaluating the botanical additives as UV protectants to improve the persistence of *Bt.* This finding will lead to prolonging the *Bt.* persistence under Indonesia's sunny and warm environments.

2. Materials and Methods

2.1. Collection and Mass Rearing of Tobacco Armyworm

This research was carried out in the Entomology Laboratory Faculty of Biology Gadjah Mada University Yogyakarta, Indonesia. The parental samples of tobacco armyworms at the larval stage were collected by hand picking from the infested cabbages at Kopeng, Magelang, Central Java.

The larvae were brought to the laboratory for mass rearing using a white bean-based artificial diet (Shorey and Hale 1965) with some modifications (Sutanto *et al.* 2017). Each plastic cup (d:45 mm, h:45 mm) was poured with twenty ml of artificial diet. The larvae were allowed to feed on the diet until the pupal stage. After that, the pupae were surface sterilized using 1% hypo chloride solution (Bayclin®-Regular, SC Johnson and Sons, Inc. IN) for two minutes and then washed using running tap water. The pupae were then air-dried for 30 minutes, laid in a glass jar (d:15 cm, t:15 cm) provided with tissue paper at the bottom, then covered tightly using muslin cloth tightened with rubber bands. When the adult emerged, four sheets of opaque paper folded like a fan were provided inside the jar for eggs laying substrate. A cotton ball soaked in a 5% honey solution was provided for moth feeding. The laid eggs were collected daily and surface sterilized as in the pupal stage. One-day-old 1st and 2nd larval instars from the 3rd generation were used for bioassays.

2.2. Additives Bio-insecticides Formulations

The additives used in this study were derived from thirteen species of plants such as jicama tuber (*Pachyrhizus erosus* (L.) Urb.), the rhizome of mango ginger (*Curcuma mangga* Val.), turmeric rhizome (*Curcuma longa* Linn.), cloves flower (*Syzygium aromaticum* (L.) Merrill and Perry), "Malang" green apple whole fruit (*Malus sylvestris* Mill.), betel leave (*Piper betle* L.), Celebes pepper (*Piper ornatum* N.E.Br.), green cabbage leave (*Brassica oleracea* L.), white cauliflower (*Brassica oleracea* L.), green cauliflower (*Brassica oleracea* L.), red cauliflower (*Brassica oleracea* L.), carrot tuber (Daucus carota (Hoffm.) Schubl and G. Martens), and Moringa leave (*Moringa oleifera* L.). These

materials were extracted based on the methods described by Sutanto *et al.* (2017), and the crude extracts were formulated with *Bacillus thuringiensis* var. kurstaki (DIPEL® WP, Abbot Comp., IN).

Two hundred grams of each plant material were cut into small pieces, added with distillate water up to 2,000 ml (10% w /v), and blended using a commercial blender. The mixture was then filtered using four layers of muslin cloth and kept at 4°C as a stock solution.

2.3. Additives Screening using UV Spectrophotometry

The UV spectrophotometry analysis used a five percent (v/v) concentration of each additive. These solutions were made by diluting 5 ml of 10% stock solution with autoclaved distillate water into 10 ml final volume. One ml of solution was used for the absorbency analysis at 190–420 nm wavelength (UV Vis, Thermo Scientific). Five additives that had the highest absorbency were selected for the Bt. formulations bioassay (Sutanto *et al.* 2017).

2.4. Pathogenicity of *Bt.* against Tobacco Armyworm

Five serial concentrations of *Bt.* 0, 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7 (spores/ml) as much as 10 ml each were prepared using autoclaved distillate water for the bioassay. Autoclaved distillate water was used as a control. One ml of the solution was poured homogeneously on the surface of twenty ml of artificial diet onto a sterile disposable petri dish (d:90 mm, h:15 mm). After that, it was air-dried at room temperature for one hour until no solution dropped on the diet surface. Then, three replicates with each of twenty-five of one-day-old first instar larvae were introduced onto the diet. The mortality of larvae was observed daily up to seven days after treatment for calculating the LC_{50} and LC_{95} (Sukirno *et al.* 2018).

2.5. The Protection Effectivity of Additives for *Bt.* against UV B Exposures

Twenty ml of additive stock solutions were taken and mixed with autoclaved distillate water up to 100 ml (2% v/v). These solutions were then used for making 100XLC_{95} *Bt.* The formula was then exposed under UV B for 0, 72, and 144 h. Each treatment used 3 replicates, and after respective exposure, the Petri dishes were kept at 4°C. After the 144-h treatment, all the UV-treated samples were added with 10 ml autoclaved distillate water to get an LC_{95} final concentration. The pathogenicity of the UV-treated *Bt.* was then tested against cotton armyworm larvae using one-day-old of 1st and 2nd larval instar (Sutanto *et al.* 2017; Sukirno *et al.* 2018). The procedure and observation of the bioassay were the same as in the preliminary bioassay.

2.6. Experimental Design and Statistical Analysis

The experimentations used a complete randomized design. The means of mortality of the treatments were analyzed using analysis of variance (ANOVA) at alpha 0.05, and the means were separated using Tukey's HSD. The pathogenicity of the *Bt.* was calculated using probit analysis. All the procedures of the analyses were using SPSS 13.

3. Results

3.1. Screening Natural Extracts with UV Spectrophotometric Methods

The absorbance of the extracts was measured as an indicator of the UV's protectant potency. From the results of the absorbance (Figure 1), there were five extracts that have the highest absorbance of UV light, especially UV B (280-320 nm) and UV C (<280 nm). The absorbance of clove extract with a range in UV-B 1.76-3.0 Od, Celebes pepper 1.040-3.0 Od, Jicama 0.184-3.0 Od, Moringa leaves 1.091-3.0 Od, and turmeric 0.475-3.0 Od. These indicated that the extracts could absorb UV light spectra.

3.2. Pathogenicity of *Bt.* against Tobacco Armyworm

Table 1 shows the pathogenicity of *Bt.* against the first larval instar of tobacco armyworm. The result indicated that the 1st larval instar was susceptible to *Bt.* The mortality of the larvae at 24 h after the treatment was significant and ranged between 69-96% at 10^2 and 10^7 spores/ml, respectively. The treatments of 10^5, 10^6, and 10^7 spores/ml caused 100% mortality after five days, while other treatments did not reach 100%. The LC_{50} (5.2 x 10^5 spores/ml) and LC_{95} (1.06 x 10^7 spores/ml) was reached at 48 h after treatment. This suggests that the application of *Bt.* is very effective for managing tobacco armyworms at the early instar.

3.3. The Pathogenicity of *Bt.* Formulations after Being Exposed to UV B against the First Larval Instar

The effects of the UV B lights at different exposure periods on the *Bt.* formulations against armyworms are depicted in Table 2. At 24 h after the treatment under unexposed UV B, the mortality of armyworm in *Bt.* formulated with Moringa leave extract was higher (82.7%) than *Bt.* alone (60%). At the 48 h after treatment, armyworm mortality in the *Bt.* alone, Moringa, Jicama, and clove formulations were the

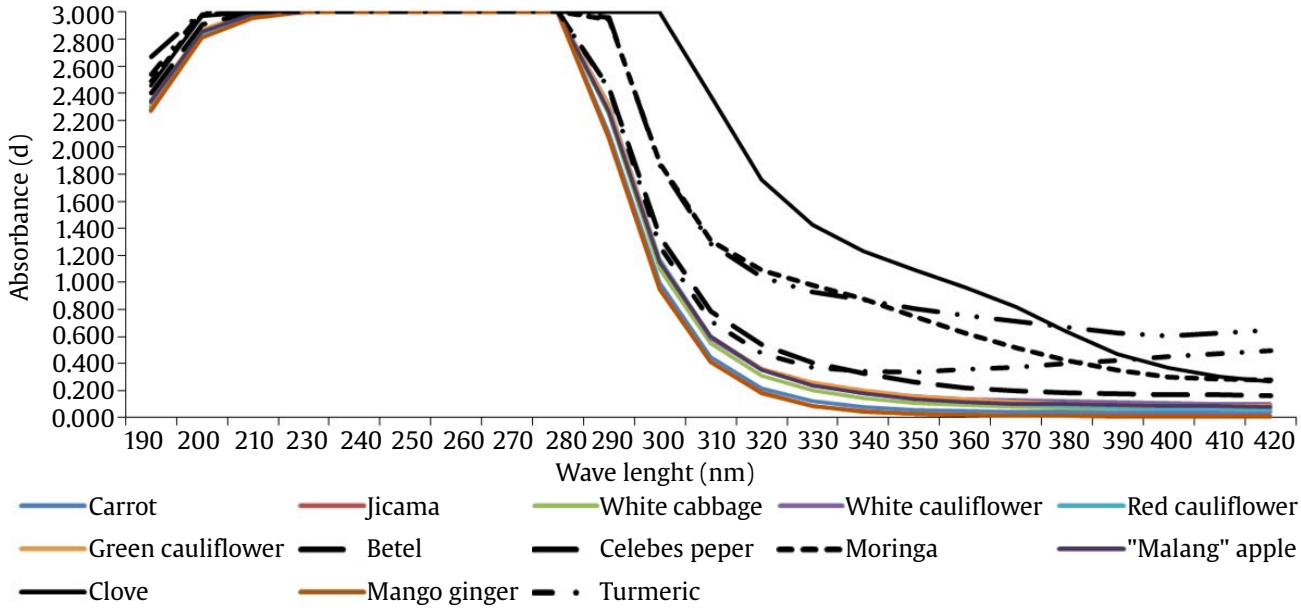

Figure 1. The absorbance of 13 of plant extracts in UV light spectra (λ: 190-420 nm)

Table 1. The mortality of first larval instar of armyworm after one to seven days of the treatments of *Bacillus thuringiensis* var. *kurstaki*

Treatment (Spores/ml)	Mortality							Statistics
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
1 x 10^2	69.3±3.5aA	85.3±7.1aAB	85.3±7.1aAB	85.3±7.1aAB	85.3±7. 1aAB	86.7±3.5aAB	89.3±3.5aAB	F = 3.18; df = 6.14; P = 0.035
1 x 10^3	84.0±2.3bA	92.0±2.3aB	92.0±2.3aB	92.0±2.3aB	92.0±2.3abB	92.0±2.3abB	93.3±1.3abB	F = 7.79; df = 6.14; P = 0.019
1 x 10^4	85.3±5.3bA	94.7±3.5aA	94.7±3.5aA	94.7±3.5aA	94.7±3.5abA	94.7±3.5abA	94.7±3.5abA	F = 1.25; df = 6.14; P = 0.342
1 x 10^5	85.3±6.1bA	98.7±1.3aB	98.7±1.3aB	98.7±1.3aB	100±0bB	100±0bB	100±0bB	F = 11.2; df = 6.14; P<0.001
1 x 10^6	92.0±6.9bA	100±0aB	100±0aB	100±0aB	100±0bB	100±0bB	100±0bB	F = 4.0; df = 6.14; P = 0.015
1 x 10^7	96.00±0bA	98.7±1.3aB	100±0aB	100±0aB	100±0bB	100±0bB	100±0bB	F = 9.0; df = 6.14; P<0.001
Statistics	F = 6.69; df = 5.12; P = 0.03	F = 2.61; df = 5.12; P = 0.081	F = 2.87; df = 5.12; P = 0.062	F = 2.87; df = 5.12; P = 0.062	F = 3.14; df = 5.12; P = 0.048	F = 6.02; df = 5.12; P = 0.05	F = 4.58; df = 5,12; P = 0.015	

Pathogenicity LC_{50} = 5.2 x 10^5 spores/ml at 48 hours after application
LC_{95} = 1.06 x 10^7 spores/ml at 48 hours after application

Numbers in the same column followed by the same small letters showed no significant difference at P< 0.05, while numbers in the same rows followed by the same capital letters showed no significance difference at P<0.05

Table 2. Mortality percentage of first larval instar of armyworm after treated with *Bt.* formulations without UV B lights exposure

Formulation	Mortality 0 h exposure (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Clove	56±2.3b	100±0c	100±0c	100±0b	100±0b	100±0b	100±0b
Celebes pepper	53.3±5.3b	90.7±2.7b	90.7±2.7b	96±4b	96±4b	96±4b	96±4b
Jicama	57.3±10.4bc	100±0c	100±0c	100±0b	100±0b	100±0b	100±0b
Moringa	82.7±8.7d	100±0c	100±0c	100±0b	100±0b	100±0b	100±0b
Turmeric	77.3±4.8cd	98.7±1.3c	98.7±1.3c	100±0b	100±0b	100±0b	100±0b
Alone	60±6.1bc	100±0c	100±0c	100±0b	100±0b	100±0b	100±0b
Control	2.7±2.7a	4±2.3a	8±2.3a	8±2.3a	8±2.3a	8±2.3a	8±2.3a

Numbers in the same column followed by the same small letters showed no significant difference at P<0.05

highest. Celebes pepper leaves did not cause 100% larval mortality. These data suggest that adding Moringa leave extract increased the pathogenicity of *Bt*. at the earlier period after the treatment compared to *Bt*. alone.

Table 3 shows the mortality of armyworm after being treated with *Bt.* formulations that were exposed for 72 h under UV B lights. The exposure to UV B lights decreased the *Bt*. pathogenicity when applied against armyworm larvae. At 1 d after the treatments, the armyworm larvae mortality in jicama was 10.7%, while *Bt*. alone was the highest (45.3%). Whereas, at 2 d after the treatment, the armyworm mortality in jicama was the lowest (32%), while the turmeric was the highest (78.7%). At 3 d after treatment, only *Bt*. formulated with Celebes pepper which had 100% mortality. There was no significant difference in the armyworm mortality at 1 to 7 d after treatments of *Bt.* formulations.

Table 4 shows the effect of *Bt*. formulations after exposure under UV B lights for 144 h on the mortality of armyworm. On 1 d after exposure, all treatments were not significantly different when compared to *Bt*. alone. Compared to 0 and 72 h UV B lights exposures, the 144 h treatments showed the pathogenicity of *Bt.* formulations at 1 d after treatment were lower. The mortality of armyworm in *Bt*. alone was the lowest (5.3%), while the formulations of Moringa and Celebes pepper were higher. At 2 d after treatments, the mortality of armyworm in *Bt*. formulations and *Bt.* alone was no significant difference. Whereas at 3 d after treatments, the mortality of armyworm in *Bt.* formulated with clove and Celebes pepper was at maximum (100%), while others did not. *Bt.* formulations and *Bt.* alone caused 100% mortality at 4 d after treatments. It indicated that there was no significant difference in pathogenicity between the *Bt.* added and *Bt.* alone treatments when applied to 1st larval instar.

3.4. The Pathogenicity of Bt. Formulations after Being Exposed under UV B against 2nd Larval Instar

UV exposed *Bt.* formulated with turmeric and Celebes pepper were then tested against 2nd larval instar of armyworm, and the mortality is depicted in Figure 2. The mortality of armyworm at 1 d after treatment showed no significant difference between *Bt.* alone and *Bt.* added formulations. Whereas, at 2 to 7 d after treatment, the mortality in *Bt.* formulated with Celebes pepper was the highest (97.3%) and the significant difference compared to *Bt.* alone. The armyworm mortality in *Bt.* alone and *Bt.* formulated with turmeric was 67 and 80%, respectively. This data showed that adding turmeric and Celebes pepper increased the pathogenicity of *Bt.* Although after being exposed to UV B lights for 72 h.

Table 3. Mortality percentage of first larval instar of armyworm after treated with *Bt.* formulations after exposed under UV light for 72 hours

Formulation	Mortality 72 h exposure (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Clove	18.7±11.6ab	42.7±7.4b	93.3±6.7b	98.7±1.3b	100±0b	100±0b	100±0b
Celebes pepper	29.3±4.8b	61.3±9.3bc	100±0b	100±0b	100±0b	100±0b	100±0b
Jicama	10.7±7.1ab	32±8.3b	98.7±1.3b	98.7±1.3b	98.7±1.3b	98.7±1.3b	98.7±1.3b
Moringa	30.7±4.8b	34.7±4.8b	98.7±1.3b	100±0b	100±0b	100±0b	100±0b
Turmeric	44±6.9b	78.7±11.4c	97.3±2.7b	97.3±2.7b	97.3±2.7b	97.3±2.7b	97.3±2.7b
Alone	45.3±23.1b	62.7±23.3bc	97.3±1.3b	100±0b	100±0b	100±0b	100±0b
Control	2.7±2.7a	4±2.3a	8±2.3a	8±2.3a	8±2.3a	8±2.3a	8±2.3a

Numbers in the same column followed by the same small letters showed no significant difference at P< 0.05

Table 4. Mortality percentage of first larval instar of armyworm after treated with *Bt.* formulations after exposed under UV light for 144 hours

Formulation	Mortality 144 h exposure (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Clove	6.7±3.5a	76±6.9b	100±0b	100±0b	100±0b	100±0b	100±0b
Celebes pepper	12±6.1a	80±2.3b	100±0b	100±0b	100±0b	100±0b	100±0b
Jicama	12±12a	69.3±9.3b	97.3±2.7b	100±0b	100±0b	100±0b	100±0b
Moringa	12±6.1a	80±12.2b	96±4b	100±0b	100±0b	100±0b	100±0b
Turmeric	8±2.3a	78.7±7.4b	97.3±2.7b	100±0b	100±0b	100±0b	100±0b
Alone	5.3±3.5a	84±2.3b	98.7±1.3b	100±0b	100±0b	100±0b	100±0b
Control	2.7±2.7a	4±2.3a	8±2.3a	8±2.3a	8±2.3a	8±2.3a	8±2.3a

Numbers in the same column followed by the same small letters showed no significant difference at P< 0.05

The effect of 144 h UV B lights exposure to the pathogenicity of *Bt.* formulations against the 2nd larval instar of armyworm are depicted in Figure 3. The mortality of armyworm at 1 d after treatment of *Bt.* formulated with Celebes pepper was the highest (38.7%), while the mortality in turmeric, *Bt.* alone, and control was 10.7, 24, and 0%, respectively. At 2 d after treatment, the mortality of armyworm in

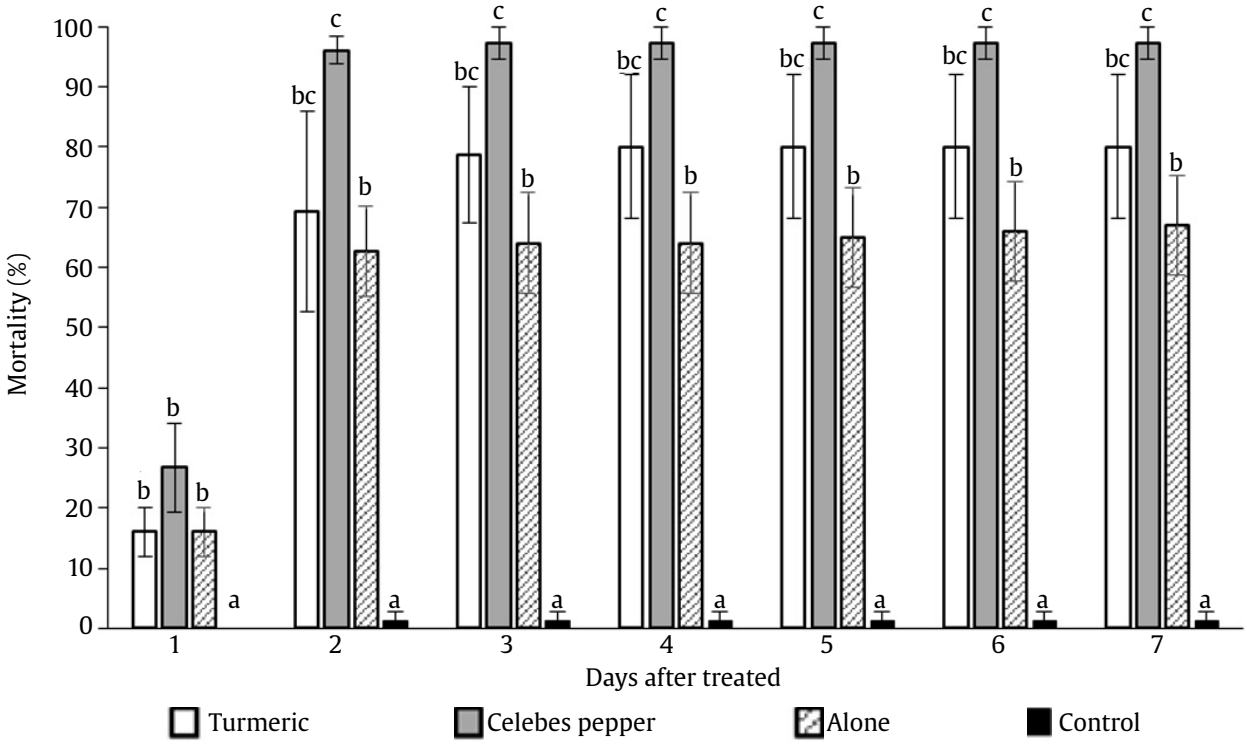

Figure 2. Mortality percentage of 2nd larval instar of armyworm after treated with *Bt.* formulations after exposed under UV for 72 hours

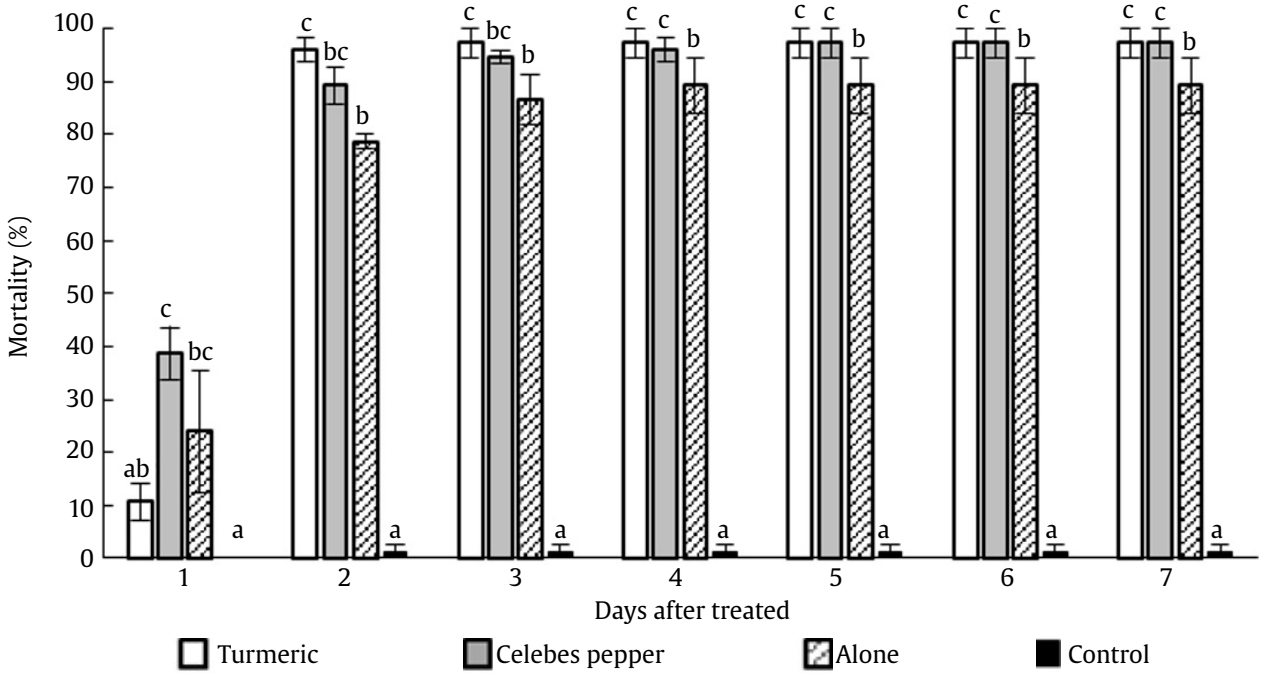

Figure 3. Mortality percentage of 2nd larval instar of armyworm after treated with *Bt.* formulations after exposed under UV for 144 hours

turmeric added *Bt.* was the highest (96%), and there was a significant difference from that in *Bt.* alone (78.7%). But, the mortality in Celebes pepper (89.3%) treatment was not significant compared to *Bt.* alone. The mortality of armyworm in *Bt.* formulated with turmeric and Celebes pepper at 4 to 7 d after treatment is not significant (97.3%) but significantly higher than in *Bt.* alone and control. This data showed that adding turmeric and Celebes pepper increased the *Bt.* pathogenicity against 2nd larval instar although it has been exposed for 144 h under UV B lights.

The pathogenicity comparison of the *Bt.* formulations against 1st and 2nd larval instar of armyworms showed that the application of *Bt.* formulated with turmeric and Celebes pepper enhanced the *Bt.* pathogenicity when compared to *Bt.* alone. The data also showed that the mortality in turmeric added *Bt.* and *Bt.* alone in 2nd instar were higher than those on earlier larval stage.

4. Discussion

The screening of additives under UV spectrophotometry showed that they could absorb UV B and UV C (Figure 1). As for spices, cloves contain more antioxidants than other common spices such as ginger, garlic, and onion. It was reported that the antioxidant in clove remains high although it has been boiled for 30 minutes (Shobana and Naidu 2000). Whereas Celebes pepper is rich in flavonoids, tannins, and alkaloids (Safithri and Fahma 2008), Moringa leaves are rich in quercetin and kaempferol (Siddhuraju and Becker 2003; Singh *et al.* 2009) which are potential as antioxidants. While, Jicama roots contain 11 compounds consisting of isoflavonoids and pterocarpan, which have UV absorption and antioxidative activities (Lukitaningsih 2014). In comparison, turmeric is rich in curcuminoids such as curcumin, bisdemethoxycurcumin, and demethoxycurcumin, which have antioxidative properties (Jayaprakasha *et al.* 2006). These suggest that clove, Jicama, Celebes betel, turmeric, and Moringa can potentially be used as UV absorbers. In this study, plant materials were extracted using water as it is simple, cheap, and can be done by the farmers. This extraction of clove might produce water-soluble compounds with antioxidant activities, such as polyphenol (Mohan *et al.* 2019) and proteins (Li *et al.* 2017). Phenolic compound (Frary *et al.* 2008) and chitosan (Long *et al.* 2018) might be produced by Celebes pepper when extracted using water as a solvent. When Moringa was extracted using water, it might contain lectin (Freitas *et al.* 2016). Tobacco armyworm has developed resistance against chemical insecticides. Many resistant tobacco armyworm strains have been found, for example, in India (Armes *et al.* 1997), Pakistan (Ahmad *et al.* 2007, 2008; Ahmad and Mehmood 2015; Shad *et al.* 2012), China (Huang *et al.* 2019; Tong *et al.* 2013), and Indonesia (Endo *et al.* 1989; Fattah *et al.* 2020). Table 1 shows that tobacco armyworm at the 1st larval instar is very sensitive to *Bt.* applications. At 1d after application, the mortality level in 10^3 (spores/ml) treatment is very effective and comparable to that of 10^4-10^7 (spores/ml) treatments. Other studies also showed that the *Bt.* is effective for tobacco armyworm control. For example, Firake and Rachna (2009) reported that the LC_{50} of *Bt.* was 0.188%. Other studies in Indonesia showed that the treatment of 10 g/L might suppress tobacco armyworm up to 75% (Rizali 2020), whereas the treatment of 15–90 ppm *Bt.* toxins had more than 50% effectivity (Pratiwi *et al.* 2016). Tables 2, 3, and 4 showed that UV B exposures were no significant effect on *Bt.* pathogenicity when applied to the 1st larval instar of tobacco armyworm. These suggested that using *Bt.* for controlling the early larval instar of tobacco armyworm using *Bt.* in Indonesia is promising as it is very susceptible.

Figures 2 and 3 showed the effect of UV B exposures on *Bt.* pathogenicity when applied to the 2nd larval instar of tobacco armyworm. At these treatments, adding Celebes pepper and turmeric significantly increased the *Bt.* pathogenicity. This showed that the addition of UV protectant effective to be applied in *Bt.* to control the later larval instar compared to 1st larval instar. The study by Jayanthi and Padmavathamma (1997) also showed that at early instar was more susceptible compared to the later instar. Another study showed that turmeric compounds also might cause the inhibition of pupation and growth (Ali *et al.* 2014). Besides that, *Bt.* alone can cause sub-lethal effects on larvae, but only temporary. The antioxidants in the additives might protect *Bt.* from free radicals produced by UV B light exposure. The *Bt.* contains Cry protein toxins which have a mode of action by disrupting the mid-gut membranes (Vachon *et al.* 2004). The later larval stages have greater feeding capability, allowing greater numbers of toxins to enter the alimentary channel, thus resulting in higher pathogenicity.

Several approaches have been made to enhance the effectiveness of Bt. for insect pest management. For example, using fusants (Revathi *et al.* 2014), adding synergistic materials (Rajguru and Sharma 2012), finding new *Bt.* isolates (Lalitha and Muralikrishna 2012; Kim *et al.* 2008; Whitlock *et al.* 1991), developing a new formulation (Devi *et al.* 2021), reducing *Bt.* particle size (Vineela *et al.* 2017), optimizing the fermentation (Amin *et al.* 2016; Sukmadi *et al.* 1999), using parasitoid synergism (Sayed *et al.* 2015), developing *Bt.* recombination (Alotaibi 2013), developing *Bt*-gene genetically modified organism (Zhang *et al.* 2006), and adding synergistic insecticides (Jayanthi and Padmavathamma 2006; Maqsood *et al.* 2019). In this study, using plant material extracts as additives is quite simple and cheaper than other approaches. It is also feasible to be applied by farmers. Thus it might stimulate them to use biological agents for integrated pest management.

The present results conclusively showed that the tobacco armyworm is susceptible to *Bt.* application. The Celebes pepper and turmeric had anti UV B and UV C properties. These extracts can potentially be used as UV B protectants for *Bt.* as components for tobacco armyworm IPM.

Conflict of Interest

All the authors declared that there was no conflict of interest.

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References

Abbaszadeh, G., Dhillon, M.K., Srivastava, C., Gautam, R.D., 2011. Effect of climatic factors on bioefficacy of biopesticides in insect pest management. *Biopestic. Int.* 7, 1-14.

Ahmad, M., Sayyed, A.H., Saleem, M.A., Ahmad, M., 2008. Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. *Crop Prot.* 27, 1367-1372. https://doi.org/10.1016/j.cropro.2008.05.003

Ahmad, M., Arif, M.I., Ahmad, M., 2007. Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *Crop Prot.* 26, 809-817. https://doi.org/10.1016/j.cropro.2006.07.006

Ahmad, M., Mehmood, R., 2015. Monitoring of resistance to new chemistry insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *J. Econ. Entomol.* 108, 1279-1288. https://doi.org/10.1093/jee/tov085

Ali, S., Sagheer, M., Hassan, M., Abbas, M., Hafeez, F., Farooq, M., Hussain, D., Saleem, M., Ghaffar, A., 2014. Insecticidal activity of turmeric (*Curcuma longa*) and garlic (*Allium sativum*) extracts against red flour beetle, *Tribolium castaneum*: a safe alternative to insecticides in stored commodities. *J. Entomol. Zool. Stud.* 2, 201-205.

Alotaibi, S.A., 2013. Mortality responses of *Spodoptera litura* following feeding on BT-sprayed plants. *J. Basic App. Sci.* 9, 195 -215. https://doi.org/10.6000/1927-5129.2013.09.27

Amin, R., Khatri, K., Panpatte, D., Pathak, L., Patel, A., Parvez, N., Shelat, H., Jani, J., Vyas, R., 2016. Optimization of fermentation parameters and *in vitro* efficacy of native *Bacillus thuringiensis* isolates against *Spodoptera litura*, in: Chakravarthy, A., Sridhara, S. (Eds.), *Arthropod Diversity and Conservation in the Tropics and Sub-tropics*. Springer, Singapore. pp. 415-435. https://doi.org/10.1007/978-981-10-1518-2_26

Armes, N.J., Wightman, J.A., Jadhav, DR, Ranga Rao, G.V., 1997. Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh, India. *Pest. Sci.* 50, 240-248. https://doi.org/10.1002/(SICI)1096-9063(199707)50:3<240::AID-PS579>3.0.CO;2-9

BALITKABI, 2015. Pengendalian larva ulat grayak (*Spodoptera litura*) dengan virus SlNPV. Buletin Balai Penelitian Tanaman Aneka Kacang dan Umbi. Available at: http://balitkabi.litbang.pertanian.go.id/infotek/pengendalian-larva-ulat-grayak-spodoptera-litura-dengan-virus-slnpv/). [Date accessed: 13 August 2015]

BPPTP, 2015. Ancaman serangan ulat grayak *Spodoptera litura* Fabricius terhadap produktifitas kedelai. Available at: http://pangan.litbang.pertanian.go.id/berita-630-ancaman-serangan-ulat-grayak-spodoptera-litura-fabricius-terhadap-produktivitas-kedelai--.html). [Date accessed: 9 April 2015]

CABI, 2018. *Spodoptera litura* (taro caterpillar). CABI datasheet. Available at: https://www.cabi.org/isc/datasheet/44520. [Date accessed: 9 October 2019]

De Oliveira, J.L., Fraceto, L.F., Bravo, A., Polanczyk, R.A., 2021. Encapsulation strategies for *Bacillus thuringiensis*: from now to the future. *J. Agric. Food Chem.* 69, 4564-4577. https://doi.org/10.1021/acs.jafc.0c07118

Devi, V.P.S., Duraimurugan, P., Chandrika, K.S.V.P., Vineela, V., 2021. Development of a water dispersible granule (WDG) formulation of *Bacillus thuringiensis* for the management of *Spodoptera litura* (Fab.). *Biocontrol Sci. Technol.* 31, 850-864. https://doi.org/10.1080/09583157.2021.1895073

do Nascimento, J., Goncalves, K.C., Dias, N.P., de Oliveira, J.L., Bravo, A., Polanczyk, R.A., 2022. Adoption of *Bacillus thuringiensis*-based biopesticides in agricultural systems and new approaches to improve their use in Brazil. *Biol. Contr.* 165, 104792. https://doi.org/10.1016/j.biocontrol.2021.104792

Dunkle, R.L., Shasha, B.S., 1989. Response of starch-encapsulated *Bacillus thuringiensis* containing ultraviolet screens to sunlight. *Environ. Entomol.* 18, 1035-1041. https://doi.org/10.1093/ee/18.6.1035

El Salamouny, S., Huber J., 2004. Effect of lignin compounds as UV protectants for *Spodoptera littoralis* nucleopolyhedrovirus. *J. Agric. Sci. Mansoura Univ.* 29, 2902-2893. https://doi.org/10.21608/jppp.2004.239721

El Salamouny, S., Ranwala, D., Shapiro, M., Shepard, B.M., Farrar B.R., 2009a. Tea, coffee, and cocoa as ultraviolet radiation protectants for the beet armyworm nucleopolyhedrovirus. *J. Econ. Entomol.* 102, 1767-1773. https://doi.org/10.1603/029.102.0506

El Salamouny, S., Shapiro, M., Ling, K.S., Shepard, B.M., 2009b. Black tea and lignin as ultraviolet protectants for the beet armyworm nucleopolyhedrovirus. *J. Entomol. Sci.* 44, 1-9. https://doi.org/10.18474/0749-8004-44.1.50

Elnagar, S., El-Sheikh, M.A.K., El Salamouny, S., Amin, A., Khattab, M., 2003. Screening of four lignin products as UV protectants to baculovirus. *Bull. Ent. Soc. Egypt.* 29, 165-178.

Endo, S., Samudra, I.M., Nugraha, A., Soejitno, J., Okada, T., 1989. Insecticidal susceptibility of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) larvae collected from three locations in Indonesia. *App. Entomol. Zool.* 24, 309-313. https://doi.org/10.1303/aez.24.309

Fattah, A., Ilyas, A., Salim, S., Djamaluddin, I. Syukur, A., 2020. Effect of several types of insecticide on predator population and damage intensity due to soybean pest attack in South Sulawesi, Indonesia. *J. Agric. Crop Res.* 8, 272-277. https://doi.org/10.33495/jacr_v8i11.20.182

Firake, D.M., Rachna, P., 2009. Relative toxicity of Proclaim 5% SG (*Emamectin benzoate*) and Dipel 8L (*Bacillus thuringiensis* var. *kurstaki*) against *Spodoptera litura* (Fab.) by leaf roll method. *Curr. Bio.* 3, 445-449.

Frary, A., Keçeli, M.A., Ökmen, B., Şığva, H.Ö., Yemenicioğlu, A., Doğanlar, S., 2008. Water-soluble antioxidant potential of Turkish pepper cultivars. *HortSci.* 43, 631-636. https://doi.org/10.21273/HORTSCI.43.3.631

Freitas, J.H.E.S., de Santana, K.V., do Nascimento, A.C.C., de Paiva, S.C., de Moura, M.C., Coelho, L.C.B.B., de Oliveira, M.B.M., Paiva, P.M.G., do Nascimento, A.E., Napoleão, T.H., 2016. Evaluation of using aluminum sulfate and water-soluble *Moringa oleifera* seed lectin to reduce turbidity and toxicity of polluted stream water. *Chemosphere.* 163, 133-141. https://doi.org/10.1016/j.chemosphere.2016.08.019

Gallagher, K.D., Kenmore, P.E., Sogawa, K., 1994. Judicial use of insecticides deter planthopper outbreaks and extend the life of resistant varieties in Southeast Asian rice, in: Denno R.F., Perfect, T.J. (Eds.), Planthoppers. Springer, Boston. pp. 599-614. https://doi.org/10.1007/978-1-4615-2395-6_18

Griego, V.M., Spence, K.D., 1978. Inactivation of *Bacillus thuringiensis* spores by ultraviolet and visible light. *Appl. Environ. Microbiol.* 35, 906-910. https://doi.org/10.1128/aem.35.5.906-910.1978

Huang, Q., Wang, X., Yao, X., Gong, C., Shen, L., 2019. Effects of bistrifluron resistance on the biological traits of *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera). *Ecotoxicol.* 28, 323-332. https://doi.org/10.1007/s10646-019-02024-2

Ignoffo, C.M., Shasha, B.S., Shapiro, M., 1991. Sunlight ultraviolet protection of the *Heliothis nuclear* polyhedrosis virus through starch-encapsulation technology. *J. Invertebr. Pathol.* 57, 134-136. https://doi.org/10.1016/0022-2011(91)90053-S

Jayanthi, P.D., Padmavathamma, K., 1997. Response of tobacco caterpillar *Spodoptera litura* (Fab) to various concentrations of *Bacillus thuringiensis* subsp. *kurstaki. Pest. Res. J.* 9, 20-24.

Jayanthi, P.K., Padmavathamma, K., 2006. Joint action of microbial and chemical insecticides on *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *J. Trop. Agric.* 39, 142-144.

Jayaprakasha, G.K., Rao, L.J., Sakariah K.K., 2006. Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin. *Food Chem.* 98, 720-724. https://doi.org/10.1016/j.foodchem.2005.06.037

Revathi, K., Chandrasekaran, R., Thanigaivel, A., Kirubakaran, S.A., Senthil-Nathan, S., 2014. Biocontrol efficacy of protoplast fusants between *Bacillus thuringiensis* and *Bacillus subtilis* against *Spodoptera litura* Fabr. *Arch. Phytopathol. Plant Prot.* 47, 1365-1375. https://doi.org/10.1080/03235408.2013.840999

Kim, D.A., Kim, J.S., Kil, M.R., Paek, S.K., Choi, S.Y., Jin, D.Y., Youn, Y.N., Hwang, I.C., Yu, Y.M., 2008. Characterization of new *Bacillus thuringiensis* isolated with bioactivities to tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Korean J. App. Entomol.* 47, 87-93. https://doi.org/10.5656/KSAE.2008.47.1.087

Lalitha, C., Muralikrishna, T., 2012. Laboratory evaluation of native *Bacillus thuringiensis* isolates against *Spodoptera litura* (Fabricius). *Curr. Biotica.* 5, 428-435.

Li, L., Lin, Z.G., Wang, S., Su, X.L., Gong, H.R., Li, H.L., Hu, F.L., Zheng, H.Q., 2017. The effects of clove oil on the enzyme activity of *Varroa destructor* Anderson and Trueman (Arachnida: Acari: Varroidae). *Saudi J. Biol. Sci.* 24, 996-1000. https://doi.org/10.1016/j.sjbs.2017.01.052

Long, L.T., Tan, L.V., Boi, V.N., Trung, T.S., 2018. Antifungal activity of water-soluble chitosan against *Colletotrichum capsici* in postharvest chili pepper. *J. Food Process. Preserv.* 42, e13339. https://doi.org/10.1111/jfpp.13339

Lukitaningsih, E., 2014. Bioactive compounds in bengkoang (*Pachyrhizus erosus*) as antioxidant and tyrosinase inhibiting agents. *Indonesian J. Pharm.* 25, 68-75. https://doi.org/10.14499/indonesianjpharm25iss2pp68

Maghsoudi, S., Jalali, E., 2017. Noble UV protective agent for *Bacillus thuringiensis* based on a combination of graphene oxide and olive oil. *Sci. Rep.* 7, 1-6. https://doi.org/10.1038/s41598-017-11080-9

Mariyono, J., 2008. The impact of integrated pest management technology on insecticide use in soybean farming in Java, Indonesia: two models of demand for insecticides. *Asian J. Agric. Dev.* 5, 43-55.

Maqsood, S., Afzal, M., Haider, M.S., Khan, H.A.A., Ali, M., Ashfaq, M., Aqueel, M.A., Ullah, M.I., 2019. Effectiveness of nuclear polyhedrosis virus and *Bacillus thuringiensis* alone and in combination against *Spodoptera litura* (Fabricius). *Pak. J. Zool.* 51, 631-641. https://doi.org/10.17582/journal.pjz/2019.51.2.631.641

Mohan, R., Jose, S., Mulakkal, J., Karpinsky-Semper, D., Swick, A.G., Krishnakumar, I.M., 2019. Water-soluble polyphenol-rich clove extract lowers pre-and post-prandial blood glucose levels in healthy and prediabetic volunteers: an open-label pilot study. *BMC Complement Altern. Med.* 19, 1-9. https://doi.org/10.1186/s12906-019-2507-7

Moustafa, M.A., Saleh, M.A., Ateya, I.R., Kandil, M.A., 2018. Influence of some environmental conditions on stability and activity of *Bacillus thuringiensis* formulations against the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Egypt J. Biol. Pest Contr.* 28, 1-7. https://doi.org/10.1186/s41938-018-0064-x

Myasnik, M., Manasherob, R., Ben-Dov, E., Zaritsky, A., Margalith, Y., Barak, Z.E., 2001. Comparative sensitivity to UV-B radiation of two *Bacillus thuringiensis* subspecies and other *Bacillus* sp. *Curr. Microbiol.* 43, 140-143. https://doi.org/10.1007/s002840010276

Pan, X., Xu, Z., Li, L., Shao, E., Chen, S., Huang, T., Chen, Z., Rao, W., Huang, T., Zhang, L. Wu, S., 2017. Adsorption of insecticidal crystal protein Cry11Aa onto nano-Mg (OH) 2: effects on bioactivity and anti-ultraviolet ability. *J. Agri.c Food Chem.* 65, 9428-9434. https://doi.org/10.1021/acs.jafc.7b03410

Pratiwi, K., Trisyono, Y.A., Martono, E., 2016. The effect of *Bacillus thuringiensis* toxin Cry1A. 105 and Cry2Ab2 on the survival of the non-target pest, *Spodoptera litura. JPTI.* 20, 7-14. https://doi.org/10.22146/jpti.16618

Rajguru, M., Sharma, A.N., 2012. Comparative efficacy of plant extracts alone and in combination with *Bacillus thuringiensis* sub sp. *kurstaki* against *Spodoptera litura* Fab. larvae. *J. Biopest.* 5, 81-86.

Rizali, A., 2020. Utilization of *Bacillus thuringiensis* in controlling armyworms (*Spodoptera litura*) on tomato (*Solanum lycopersicum*) plants. *J. Biodiv. Environ. Sci.* 14, 118-123.

Safithri, M., Fahma, F., 2008. Potency of *Piper crocatum* decoction as an antihiperglycemia in rat strain Sprague Dawley. *HAYATI J. Biosci.* 15, 45-48. https://doi.org/10.4308/hjb.15.1.45

Sansinenea, E., Ortiz A., 2015. Melanin: a photoprotection for *Bacillus thuringiensis* based biopesticides. *Biotechnol. Lett.* 37, 483-490.

Sayed, S.M., Mahmoud, S.F., Elzahrany, O.M., Elsayed, G., 2015. Efficacy of *Bacillus thuringiensis* and indigenous *Trichogramma turkistanica* for controlling lepidopterous pests on *Taify pomegranate* fruits. *African Entomol.* 23, 443-450. https://doi.org/10.4001/003.023.0229

Shad, S.A., Sayyed, A.H., Fazal, S., Saleem, M.A., Zaka, S.M., Ali, M., 2012. Field evolved resistance to carbamates, organophosphates, pyrethroids, and new chemistry insecticides in *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). *J. Pest Sci.* 85, 153-162. https://doi.org/10.1007/s10340-011-0404-z

Shapiro, M., El Salamouny, S., Shepard, B.M., Jackson, D.M., 2009a. Plant phenolics as radiation protectants for the beet armyworm (Lepidoptera: Noctuidae) nucleopolyhedrovirus. *J. Agric. Urban Entomol.* 26, 1-10. https://doi.org/10.3954/1523-5475-26.1.1

Shapiro, M., El Salamouny, S., Shepard, B.M., 2009b. Plant extracts as ultraviolet radiation protectants for the beet armyworm (Lepidoptera: Noctuidae) nucleopolyhedrovirus: screening of extracts. *J. Agric. Urban. Entomol.* 26, 47-61. https://doi.org/10.3954/1523-5475-26.2.47

Shapiro, M., El Salamouny, S., Shepard, M., 2008. Green tea extracts as ultraviolet protectants for the beet armyworm, *Spodoptera exigua* nucleopolyhedrovirus. *J. Bio. Sci. Technol.* 18, 605-617. https://doi.org/10.1080/09583150802133271

Shapiro, M., Farrar, R., Domex, J., Javaid, I., 2002. Effects of virus concentration and ultraviolet irradiation on the activity of corn earworm and beet armyworm (Lepidoptera: Noctuidae) nucleopolyhedroviruses. *J. Econ. Entomol.* 95, 243-249. https://doi.org/10.1603/0022-0493-95.2.243

Shapiro, M., Robertson, J.L., 1990. Laboratory evaluation of dyes as ultraviolet screen for the gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. *J. Econ. Entomol.* 83, 168-172. https://doi.org/10.1093/jee/83.1.168

Shepard, B.M., Shapiro, M., El Salamouny, S., 2010. Plant derived protectants against ultraviolet light. US Patent Nr. US2010/0215630A1

Shorey, H.H., Hale, R.L., 1965. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. *J. Econ. Entomol.* 58, 522-524. https://doi.org/10.1093/jee/58.3.522

Singh, B.N., Singh, B.R., Singh, R.L., Prakash, D., Dhakarey, R., Upadhyay, G., Singh, H.B., 2009. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera. Food Chem. Toxicol.* 47, 1109-1116. https://doi.org/10.1016/j.fct.2009.01.034

Siddhuraju, P., Becker, K., 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J. Agric. Food Chem.* 51, 2144-2155. https://doi.org/10.1021/jf020444+

Sukirno, S., Tufail, M., Rasool, KG, El Salamouny, S., Sutanto, K.D., Aldawood, A.S., 2017. The effectiveness of spinosad and neem extract against *Spodoptera littoralis* (BOISD.) and *Spodoptera exigua* (Hubner): exploring possibilities to enhance the bio-pesticide persistence with natural UV protectants under field-sunlight condition of Saudi Arabia. *Pak. J. Agric. Sci.* 54, 743-751. https://doi.org/10.21162/PAKJAS/17.5306

Sukirno, S., Tufail, M., Rasool, KG, El Salamouny, S., Sutanto, K.D., Aldawood, A.S., 2018. The efficacy and persistence of *Spodoptera littoralis* nucleopolyhedrovirus (SpliMNPV) applied in UV protectants against the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) under Saudi field conditions. *Pak. J. Zool.* 50, 1895-1902. https://doi.org/10.17582/journal.pjz/2018.50.5.1895.1902

Sukmadi, B., Permatasari, A.U., Hadioetomo, R.S., 1999. The use of technical grade glucose as the carbon sources in the fermentation media of *Bacillus thuringiensis* subsp. Aizawai. In: Seminar Pertemuan Ilmiah Tahunan Perhimpunan Mikrobiologi Indonesia, Bandar Lampung (Indonesia), 14-15 Dec 1998. PERMI Cabang Lampung.

Sutanto, K.D., El Salamouny, S., Tufail, M., Rasool, K.G., Sukirno, S., Shepard, M., Shapiro, M., Aldawood, A.S., 2017. Evaluation of natural additives to enhance the persistence of *Spodoptera littoralis* (Lepidoptera: Noctuidae) nucleopolyhedrovirus (SpliMNPV) under field conditions in Saudi Arabia. *J. Econ. Entomol.* 110, 924-930. https://doi.org/10.1093/jee/tox085

Shobana, S., Naidu, K.A., 2000. Antioxidant activity of selected Indian spices. *PLEFA.* 62, 107-110. https://doi.org/10.1054/plef.1999.0128

Tamez-Guerra, P., McGuire, M.R., Behle, R.W., Hamm, J.J., Sumner, H.R., Shasham B.S., 2000. Sunlight persistence and rain fastness of spray-dried formulations of baculovirus isolated from *Anagrapha falcifera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 93, 210-218. https://doi.org/10.1603/0022-0493-93.2.210

Tong, H., Su, Q., Zhou, X., Bai, L., 2013. Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. *J. Pest Sci.* 86, 599-609. https://doi.org/10.1007/s10340-013-0505-y

Vachon, V., Prefontaine, G., Rang, C., Coux, F., Juteau, M., Schwartz, J.L., Brousseau, R., Frutos, R., Laprade, R., Masson, L., 2004. Helix 4 mutants of the *Bacillus thuringiensis* insecticidal toxin Cry1Aa display altered pore-forming abilities. *Appl. Environ. Microbiol.* 70, 6123-6130. https://doi.org/10.1128/AEM.70.10.6123-6130.2004

Van Bokhorst-van de Veen, H., Xie, H., Esveld, E., Abee, T., Mastwijk, H., Groot, M.N., 2015. Inactivation of chemical and heat-resistant spores of *Bacillus* and *Geobacillus* by nitrogen cold atmospheric plasma evokes distinct changes in morphology and integrity of spores. *Food Microbiol.* 45, 26-33. https://doi.org/10.1016/j.fm.2014.03.018

Vineela, V., Nataraj, T., Reddy, G., Devi, V.P.S., 2017. Enhanced bioefficacy of *Bacillus thuringiensis* var. *kurstaki* against *Spodoptera litura* (Lepidoptera: Noctuidae) through particle size reduction and formulation as a suspension concentrate. *Biocontrol Sci. Technol.* 27, 58-69. https://doi.org/10.1080/09583157.2016.1247433

Whitlock, V.H., Lo, M.C., Kuo, M.H., Soong, T.S., 1991. Two new isolates of Bacillus thuringiensis pathogenic to *Spodoptera litura. J. Invertebr. Pathol.* 58, 33-39. https://doi.org/10.1016/0022-2011(91)90159-N

Wu, L., Deng, J., Tan, X., Yin, W., Ding, F., Han, H., 2018. Ratiometric fluorescence sensor for the sensitive detection of *Bacillus thuringiensis* transgenic sequence based on silica coated super magnetic nanoparticles and quantum dots. *Sens. Actuators B Chem.* 254, 206-213. https://doi.org/10.1016/j.snb.2017.07.021

Zhang, G.F., Wan, F.H., Liu, W.X., Guo, J.Y., 2006. Early instar response to plant-delivered Bt-toxin in a herbivore (*Spodoptera litura*) and a predator (*Propylaea japonica*). *Crop Prot.* 25, 527-533. https://doi.org/10.1016/j.cropro.2005.08.008

Zhang, W.J., Bjorn, L.O., 2009. The effect of ultraviolet radiation on the accumulation of medical compound in plant. *Fitoterapia.* 80, 207-218. https://doi.org/10.1016/j.fitote.2009.02.006

Zhang, L., Zhang, X., Wu, S., Gelbic, I., Xu, L., Guan, X., 2016. A new formulation of *Bacillus thuringiensis*: UV protection and sustained release mosquito larvae studies. *Sci. Rep.* 6, 1-8. https://doi.org/10.1038/srep39425

Zhou, X., Li, H., Liu, Y., Hao, J., Liu, H., Lu, X., 2018. Improvement of stability of insecticidal proteins from *Bacillus thuringiensis* against UV-irradiation by adsorption on sepiolite. *Adsorption. Sci. Technol.* 36, 1233-1245. https://doi.org/10.1177/0263617418759777

Zogo, B., Tchiekoi, B.N.C., Koffi, A.A., Dahounto, A., Alou, L.P.A., Dabiré, R.K., Baba-Moussa, L., Moiroux, N., Pennetier, C., 2019. Impact of sunlight exposure on the residual efficacy of biolarvicides *Bacillus thuringiensis* israelensis and *Bacillus sphaericus* against the main malaria vector, Anopheles gambiae. *Malar. J.* 18, 1-9. https://doi.org/10.1186/s12936-019-2687-0