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Research article

Evaluation of Natural Additives to Enhance the Persistence of *Spodoptera littoralis* (Lepidoptera: Noctuidae) Nucleopolyhedrovirus (*Spli*MNPV) Under Field Conditions in Saudi Arabia

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Abstract

Nucleopolyhedrovirus is an effective biocontrol agent but for its biggest disadvantage of short persistence under sunlight conditions. In this study, 10 plant extracts were evaluated as ultraviolet (UV) protectants to improve the persistence of *Spodoptera littoralis* multiple-embedded nucleopolyhedrovirus (*Spli*MNPV) against cotton leafworm (*Spodoptera littoralis* Boisduval). In the primary lab screening test, 5 out of 10 additives (cloves, henna, green tea, pomegranate, and grape extracts) presented a high rate of virus protection with original activity remaining (OAR) percentage of 100%, 97%, 91%, 90.6%, and 77%, respectively, when used at a concentration of 1% and exposed to UVB for a period of 1 h. A secondary screening was then performed with these best five extracts at a concentration of 0.5% and for an exposure timing of 5 h to UVB. Among these, clove and henna that showed highest protection with OAR values of 96.6% and 76.5%, respectively, were selected for the field trials. When applied on cabbage in the field during sunny summer conditions, clove and henna extracts enhanced the persistence of *Spli*MNPV by twofold. These findings are encouraging to be applied in the field studies.

Key words: biological control, plant extract, Noctuidae, *Spli*MNPV, *Spodoptera littoralis*

The cotton leafworm, *Spodoptera littoralis* Boisduval, is one of the most destructive pests worldwide, and has a wide host spectrum ranging from crucifers, grasses, legumes to many vegetables and field crops (Ellis 2004). This pest has become the major limiting factor and threat to several field crops and vegetables production in many countries including Saudi Arabia (Albarrak 2009). For its control, the extensive use of chemical insecticides has led to the insecticide resistance. Moreover, the increasing awareness on environmental pollution and the high demand of safe food production has led to the increased interests in using alternative methods to reduce the pesticides dependency in plant protection. Baculoviruses are reported to be the promising candidates due to their eco-safety and target specificity (Burges et al. 1980). A nucleopolyhedrovirus (NPV) was first isolated from *S. littoralis* larvae in Egypt (Abul-Nasr 1956), and characterization of this virus (*Spli*MNPV) has been done (Seufi 2008). This NPV was tested under field conditions and is now commercially available in the market as Spodopterin (Ajay Bio-Tech Ltd., India) and Littovir (Biocontrol, Switzerland).

It is well documented that UV (ultraviolet) in the sunlight is the major factor depleting the persistence of microbial control agents (Jaques 1977, Ignoffo et al. 1989, Jones et al. 1993, Behle et al. 1997). There is a considerable evidence that the UVB portion (280–320 nm wavelength) of sunlight inactivates the baculoviruses (Ignoffo and Batzer 1971).

To improve the persistence of baculoviruses in the field, several chemical additives related to UV protection have already been tried

out such as fluorescent brightener (Dougherty et al. 1996), Congo red, indigo carmine (Shapiro and Robertson 1990), and the carbon (Ignoffo et al. 1991). However, in recent years, interest in the use of natural UV protectants in virus formulations has grown. For example, lignin as a side product of paper industry is a potent UV protectant to *S. littoralis* MNPV (Tamez-Guerra et al. 2000, Elnagar et al. 2003, El Salamouny and Huber 2004). Also, magnesium lignosulfonate is recognized for its ability to improve the persistence of *Helicoverpa armigera* MNPV (El Salamouny et al. 2000). Moreover, lignin in the presence of pregelatinized corn flour also enhances the activity of *Anagrafa falcifera* MNPV (Behle et al. 2003). Beverages such as black tea, green tea, cocoa, and coffee have also been shown as UV protectants for *S. exigua* MNPV (Shapiro et al. 2008, El Salamouny et al. 2009b). Also, extracts of spices, medicinal herbs, and weeds are shown to be promising UV protectants (Shapiro et al. 2009a, Shepard et al. 2010). Plant extracts are able to improve the virus persistence under the field conditions (Shapiro et al. 2008, Shapiro et al. 2012). Although several efforts have been made to identify the natural UV protectants, the scientists still search for the best one.

Most of the Kingdom of Saudi Arabia (KSA) has arid climate which has sunny dry conditions. Thus, the present study focuses on evaluating the 10 botanical additives as UV protectant to improve the persistence of *S. littoralis* multiple-embedded nucleopolyhedrovirus (*Spli*MNPV) under summer sunny conditions in KSA. Finding an effective additive will help to solve the problem of the fast inactivation of baculoviruses under the harsh sunny conditions.

Materials and Methods

Test Insect

The cotton leafworm, *S. littoralis*, females were collected using light traps placed in vegetable farms in Al-waseel, Riyadh, Saudi Arabia. The colony was maintained in the laboratory on a white beans-based semiartificial diet (Shorey and Hale 1965) under controlled conditions of 25 °C and 60–70% RH in a growth chamber (Steridium, Australia).

Virus Source

The virus source used (*Spli*MNPV) was the commercial product Littovir (Biocontrol, Switzerland). The concentration of the product was 2×10^{12} PIB/liter (polyhedral inclusion body). The required dilutions of the virus suspensions were prepared using distilled water and kept in glass tubes (Lab. Glass, India) at 4 °C until used.

Plant Natural Additives

Ten plant extracts belonging to different botanical families were evaluated to analyze their ability as UV protectant. The tested plant extracts were as follows (Order and Family in parentheses): henna whole leaves, *Lawsonia inermis* L. (Myrtales, Lythraceae); dates fruit (variety Sukari) without seed, *Phoenix dactylifera* L. (Arecales, Arecaceae); black grape whole fruit, *Vitis vinifera* L. (Vitales, Vitaceae); kiwi whole fruit, *Actinidia deliciosa* (Chev.) Liang & Ferguson (Ericales, Actinidiaceae); olive fruit without seed, *Olea europae* L. (Lamiales, Oleaceae); lemon whole fruit, *Citrus limon* (L.) Burm (Sapindales, Rutaceae); pomegranate whole fruit without skin, *Punica granatum* L. (Myrtales, Lythraceae); red beetroot whole tuber, *Beta vulgaris* L. (Caryophyllales, Amaranthaceae); Green tea whole leaves, *Camellia sinensis* (L.) Kuntze (Ericales, Theaceae); and clove whole flower, *Syzygium aromaticum* (L.) Merrill & Perry (Myrtales, Myrtaceae).

Preparation of UV Protectant Additives

Two grams of each dry plant material was soaked in 100 ml distilled water for 24 h at the room temperature to get a final stock solution. The mixture was blended and filtered through muslin cloth layers. These filtrates were added to virus inoculum to get the final concentration of *Spli*MNPV of 2.23×10^6 PIBs/ml following the method described by Shapiro et al. (2008).

Measuring the Absorbance of Tested Additives

Absorption spectra of UV radiation of the tested additives were determined using spectrophotometer (JENWAY, 6705 UV/Vis., England) in January 2013. The absorption spectra measurements were conducted by using 1 ml of each sample of fourfold dilutions of the stock solution and exposed under UV (wavelengths 190–500 nm; Shapiro et al. 2012). To confirm the reproducibility of the data, the fresh plant extracts of clove and henna that were showing the highest UV protection were re-screened for their UV absorption ability in December 2016. There was no any temporal difference observed for UV protection of both clove and henna.

Screening of Plant Extracts as UV Protectant Additives

To evaluate the selected plant extracts as UV protectants, two screening steps were conducted. In primary screening, the extract of twofold dilutions of stock solutions were exposed for 1 h to UVB. However, in case of the secondary screening, the additive concentration was reduced to fourfold dilutions while exposure time was extended to 5 h. Also, the best five extracts obtained from primary screen were further tested in the secondary screening.

Ultraviolet-B Exposure and Bioassay Under Laboratory Conditions

Two UVB lamps of 15 watt, 302 nm (Fotodyne Inc., NewBerlin, WI, Germany) were used to simulate the natural sunlight UV in the lab. The lamps were fixed in an irradiation box (ADECO, Saudi Arabia) at a height of 40 cm from top of the samples under test while samples were kept in centers of the both light sources. To evaluate the ability of plant extracts as UV protectants, comparative UVB exposure test was conducted using 4 ml of virus suspension, *Spli*MNPV of 2.23×10^6 PIBs/ml (final concentration), with or without additives by spreading in a glass petri dish (60 by 15, Duran Group, Czech). For this, three replicates were used for each sample while each replicate was placed randomly under the UVB source. After respected exposure timing, the suspensions were collected in glass tubes, volume of each suspension was adjusted up to 4 ml (the original volume) with distilled water, and then stored at 4 °C until bioassay.

The effectiveness of the additive-formulated virus after being exposed to UVB was then evaluated against *S. littoralis* first-instar larvae using diet treatment bioassay. For this, 1 ml of each virus suspension was evenly distributed onto white bean-based artificial diet in a bioassay plate (5 by 10 by 1.5 cm; LICEFA, Bad Salzuflen (DE) Germany). After that, a 50-cells bioassay rack was fixed inside this plate resulting in 50 cells of 1- by 1-cm diet in each. The diet was then allowed to dry for 2 h at room temperature. After that, 50 neonate larvae were introduced into each plate (one larva per each cell), and three replicates of each treatment were used in the bioassay. Larval mortality was observed daily up to 10 d, and the virus protection rate was measured in terms of the percentage of original activity remaining (OAR%) as reported by Shapiro et al. (2008).

Table 1. Primary screening: The comparative effectiveness and means of OAR (%) ± SE of *Spli*MNPV after being blended with 1% of 10 natural additives exposed to UVB light for 1 h, and tested against *S. littoralis* first-instar larvae

Treatments	Mortality (%)		Statistic[a]	OAR (%)
	0 h	1 h		
V alone	96.17 ± 2.08aB	0.00 ± 0.00aA	$F = 6.15$; df = 1, 4; $P = 0.01$	0.0 ± 0.0a
V + Clove	95.87 ± 2.07aA	96.42 ± 0.70dA	$F = 5.28$; df = 1, 4; $P = 0.08$	100. ± 2.4c
V + Henna	96.00 ± 3.06aA	93.24 ± 2.31dA	$F = 0.46$; df = 1, 4; $P = 0.53$	97.0 ± 2.6c
V + Green tea	99.29 ± 0.71aB	90.28 ± 5.01dA	$F = 7.42$; df = 1, 4; $P = 0.05$	91.0 ± 4.5c
V + Pomegranate	100.00 ± 0.00aB	90.69 ± 2.49dA	$F = 16.0$; df = 1, 4; $P = 0.02$	90.0 ± 4.0c
V + Grape	95.10 ± 3.28aA	73.3314.5cdA	$F = 3.36$; df = 1, 4; $P = 0.14$	77.0 ± 14bc
V + Lemon	94.20 ± 5.80aB	66.67 ± 9.39cdA	$F = 0.38$; df = 1, 4; $P = 0.05$	71.0 ± 6.4bc
V + Kiwi	97.53 ± 2.47aB	57.02 ± 22.1cA	$F = 12.09$; df = 1, 4; $P = 0.03$	58.40 ± 23b
V + Olive	79.93 ± 0.55bA	42.26 ± 7.02bcA	$F = 1.29$; df = 1, 4; $P = 0.32$	53.0 ± 8.6b
V + Date	97.33 ± 1.76aB	20.00 ± 14.0abA	$F = 11.39$; df = 1, 4; $P = 0.03$	21.00 ± 15a
V + Beet root	99.33 ± 0.67aB	17.99 ± 10.7abA	$F = 13.47$; df = 1, 4; $P = 0.02$	18.00 ± 11a
Statistic[b]	$F = 4.56$; df = 10, 22; $P = 0.001$	$F = 10.92$; df = 10, 22; $P < 0.001$	–	$F = 11.23$; df = 10, 22; $P < 0.001$

V indicates the virus. Means followed by the same capital letters in the same row are not significantly different at α: 0.05 (Statistic[a]). Means followed by the same letter (lower case) in the same column are not significantly different at α: 0.05 (Statistic[b]).

Evaluation of Clove- and Henna-Formulated *Spli*MNPV Persistence on the Cabbage Plants Under Field Conditions

The best two plant extracts (clove and henna) obtained after primary and secondary screenings for their highest protection ability to virus were then evaluated under field conditions. For this, the cabbage plants (6 wk old) cultivated under sunny-field conditions at the Educational Farm, King Saud University (Riyadh, Saudi Arabia), were applied with clove and henna at the concentration of 100 g plant extract/liter mixed with *Spli*MNPV (final concentration of 2×10^7 PIB/ml). The final volume of virus formulation (with or without additives) used for each treatment was 300 ml, and was applied by spraying on upper surface of the seven cabbage leaves using plastic hand sprayer (500 ml; Albawazir, Riyadh, Saudi Arabia). This experiment was conducted during summer (May) of 2013; three replicates were used for each treatment while the average field temperature recorded was 38 °C.

The virus-treated cabbage leaves were then collected from the experimental field on day 0, 1, 3, 5 and 7 posttreatments. The collected leaves were kept in plastic zip bags, transferred to the laboratory, and kept frozen at −20 °C until used for bioassay.

For bioassay, the frozen leaf samples were prepared by cutting with a falcon tube (diameter 4 cm). These leaf discs were then placed, 1 h prior to the bioassay, onto the agar layer (1%) prepared in small plastic cups (5 cm in diameter). This agar was used to avoid the dryness of leaf discs. A batch of 20 first-instar larvae, with three replicates, was allowed to feed on the leaf disc for 48 h, and then the larvae were transferred to an artificial diet and followed up to 10 d. Mortality was used as an indicator to detect the activity of the virus after sunlight exposure in the presence and absence of the additives.

Statistical Analysis

Probit analysis was used to calculate the lethal concentration (LC_{95}) values (Finney 1977). The original activity remaining percentage (OAR%) of each treatment was calculated to determine its potential to enhance the virus (Ignoffo and Batzer 1971) as the percentage mortality caused by virus unexposed to UV divided by percentage mortality caused by virus exposed to UV times by 100%.

The probit analysis was done by transforming the mortality percentage into log value, while OAR% was also transformed into log value for factorial analysis of variance (ANOVA) to test the differences among treatments. The probit analysis and ANOVA were carried out using complete randomized design while the means were separated using LSD test (SPSSInc 2005).

Results

Primary Screening of Plant Extracts as UV Protectants for *Spli*MNPV

Data presented in Table 1 showed a complete inactivation of *Spli*MNPV when applied alone and exposed to UV for 1 h as compared to a blend of virus with plant extracts at twofold dilutions of stock solution. The highest rate of virus protection as measured in terms of OAR% was recorded in the clove extract treatment (100%) then followed by henna, green tea, pomegranate, and grape extracts where the OAR% values observed were 97, 91, 90.6, and 77%, respectively. However, lemon and kiwi extracts revealed a moderate rate of virus protection, with OAR% of 71 and 58.4%, respectively. The lowest rate of virus protection was recorded in the treatment of olive, dates (variety Sukari), and beetroot extracts, with OAR% of 53, 21, and 18%, respectively (Table 1). Also, in olive treatment with no UV exposure, the mortality level was found considerably low (79.93%) as compared to other treatments. There is possibility that some compound in olive extract may inhibit the virus competency. The obtained preliminary screening data demonstrated that clove, henna, green tea, pomegranate, and grape extracts are the most potent candidates to enhance the virus persistence when exposed to UV (see Table 1). These five best additives were deployed for the secondary screening to further identify the most effective among them.

Secondary Screening of the Additives Selected From Primary Screening as UV Protectants for *Spli*MNPV

Secondary screening was conducted by testing five best plant extracts obtained from the primary screening to further select the best candidates for virus protection. Data presented in Table 2 showed

Table 2. Secondary screening: The comparative effectiveness and means of OAR (%) ± SE of *Spli*MNPV after being blended with 0.5% of the best five natural additives exposed to UVB light for 5 h, and tested against *S. littoralis* first-instar larvae

Treatments	Mortality (%)		Statistic[a]	OAR
	0 h	5 h		
V alone	98.59 ± 0.70aB	00.00 ± 0.00aA	$F = 14.91$; df = 1, 4; $P = 0.02$	00.0 ± 0.00a
V + Clove	96.87 ± 1.10abB	93.67 ± 4.30cA	$F = 8.34$; df = 1, 4; $P = 0.04$	96.6 ± 4.30c
V + Henna	97.67 ± 2.30aA	74.76 ± 10.5bcA	$F = 5.83$; df = 1, 4; $P = 0.07$	76.5 ± 10.0c
V + Green tea	91.54 ± 2.04bA	62.58 ± 10.2bA	$F = 3.43$; df = 1, 4; $P = 0.14$	68.3 ± 10.0b
V + Pomegranate	96.87 ± 2.18abA	64.81 ± 11.1bA	$F = 2.75$; df = 1, 4; $P = 0.17$	66.9 ± 11.0b
V + Grape	94.57 ± 1.80abB	8.55 ± 8.50aA	$F = 0.38$; df = 1, 4; $P = 0.04$	9.00 ± 8.50a
Statistic[b]	$F = 2.02$; df = 5, 12; $P = 0.15$	$F = 19.81$; df = 5, 12; $P < 0.001$	–	$F = 20.58$; df = 5, 12; $P < 0.001$

V indicates the virus. Means followed by the same capital letters in the same row are not significantly different at α: 0.05 (Statistic[a]). Means followed by the same letter (lower case) in the same column are not significantly different at α: 0.05 (Statistic[b]).

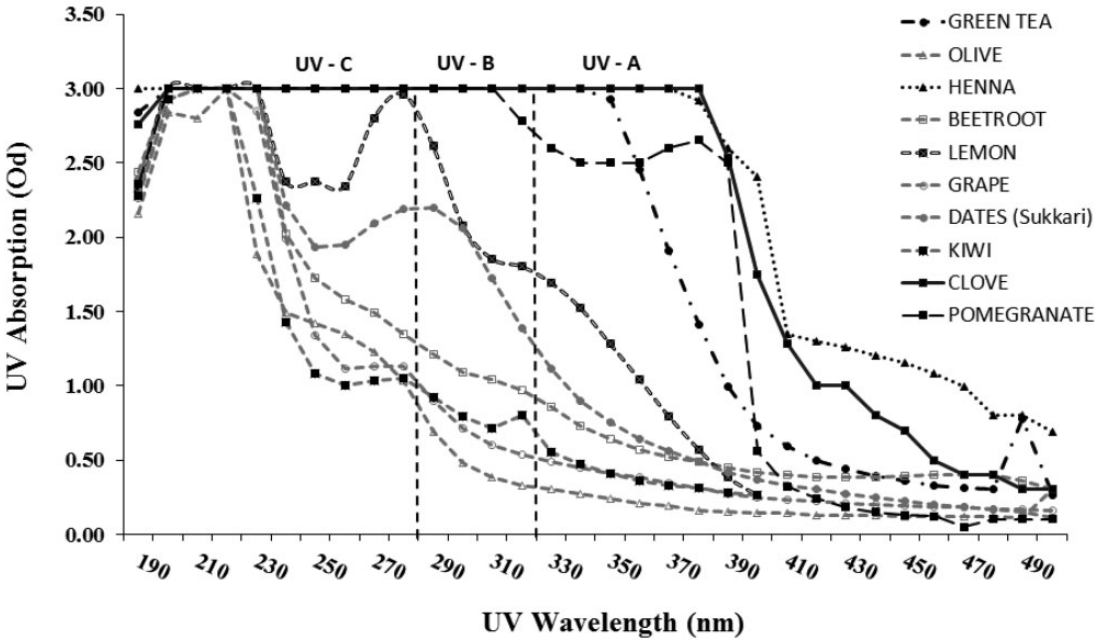

Fig. 1. The absorption spectra of green tea, olive, henna, beetroot, lemon, grape, dates, kiwi, clove, and pomegranate extracts measured by spectrophotometer at the wave length range of 190–500 nm.

that addition of clove, henna, and green tea extracts provided the highest rate of protection to *Spli*MNPV. The OAR% value in clove extract treatment was found to be 96.6%. However, in case of henna and green tea extracts, this value (OAR%) was 76.5% and 68.3%, respectively. In contrast, the pomegranate and grape extracts showed the lowest rate of protection, with OAR values of 66.9 and 9.0, respectively (Table 2). The secondary screening data again revealed that clove and henna extracts were the best additives as UV protectants for virus protection, and were thus tested under field conditions.

Absorption Spectra of Tested Additives

The absorption spectra of the tested additives support the UV protection as determined by %OAR (Fig. 1). The rates of UV absorption of clove and henna extracts were higher among all natural additives tested (Fig. 1). Moreover, the absorption spectra also indicated that clove and henna extracts had a good potential to absorb UVB (280–320 nm) as well as UV-C (<280 nm), suggesting their competency as the best candidates for UV protection. To confirm the reproducibility of the data, the fresh plant extracts of both clove and henna were re-screened for their UV absorption ability as mentioned before in Materials and Methods. There was no temporal difference observed for the UV protection capability in both these additives.

Evaluation of Persistency of Clove- and Henna-Formulated *Spli*MNPV on the Cabbage Plants Under Field Conditions

Based on laboratory screening, clove and henna extracts were finally selected to evaluate their competency as UV protectants under field conditions (Table 3). For the formulated virus that was exposed to natural sunlight, the factorial analysis showed a significant interaction of time interval and virus formulations to the OAR ($F = 3.78$; df = 5, 19; $P = 0.007$). The data showed that treatment with virus

Table 3. The average original activity remaining (OAR% ± SE) of the clove and henna as UV protectants of *Spli*MNPV tested on cabbage leaves against cotton leafworm, *S. littoralis*, first larval instar under field conditions

Time intervals (d)	Virus alone	V+ Henna	V+ Clove	Statistic[a]
0	100Ac	98Ab	94.74Aa	$F = 2.1$; df = 2, 6; $P = 0.20$
1	94.29Abc	87.93Aab	98.11Aa	$F = 2.32$; df = 2,6; $P = 0.18$
3	72.50Ab	59.18Aa	85.71Aa	$F = 2.68$; df = 2, 6; $P = 0.15$
5	42.86Aa	63.27ABa	75.56Ba	$F = 3.49$; df = 2, 6; $P = 0.09$
7	30.56Aa	66.04ABa	79.49Ba	$F = 4.97$; df = 2, 6; $P = 0.05$
Statistic[b]	$F = 13.88$; df = 4, 10; $P < 0.001$	$F = 3.25$; df = 4, 10; $P = 0.05$	$F = 2.02$; df = 4,10; $P = 0.17$	

V indicates the virus.

[a]Means with the same capital letter in different virus formulations of the same sunlight exposures are not significantly different at α: 0.05 (comparison between the columns). [b]Means with the same letter (lower case) in the different sunlight exposure treatments are not significantly different at α: 0.05 (comparison between the rows).

alone lost its activity faster than that of blended with henna or clove extracts. The OAR (%) values of the treatments with virus alone were decreased from 100% (with zero or no sunlight exposure) to 94.29%, 72.5%, 42.86%, and 30.56% when exposed for a period of 0, 1, 3, 5, and 7 d, respectively, under the field conditions. In our field trials, the OAR% value of the virus alone treatment remained only 30.56% at 7 d posttreatment.

On the other hand, the addition of natural UV protectants clove and henna extracts improved the persistence of *Spli*MNPV in the field under sunlight condition. The addition of clove extract to *Spli*MNPV formulation prolonged the virus persistence where the % OAR values recorded were 94.74%, 98.11%, 85.71%, 75.56%, and 79.49% after 0, 1, 3, 5, and 7 d of exposure, respectively, while in henna-treated virus, the OAR values were 98%, 87.93%, 59.18%, 63.27%, and 66.04%, respectively, after the same exposure timings. In the present study, clove and henna extracts showed relatively good protection after 7 d of exposure to sunlight with % OAR of 79.49 and 66.04%, respectively.

Discussion

It is well-documented that the reactive oxygen species (ROS) are destructive to the viral DNA and also affect the degradation of matrix proteins (Bandyopadhyay et al. 1999). It was reported that UV damage the DNA by producing two types of pyrimidine dimers—cyclobutane pyrimidine dimers and pyrimidine–pyrimidine 6–4 photoproducts (Friedberg et al. 1995). The selection of natural additives in present study was based on the hypothesis of having high antioxidant contents. This antioxidant could prevent the pyrimidine dimer formation. The finding of clove and henna extracts as good UV protectants is consistent with Shapiro et al. (2008) and Shapiro et al. (2009a), where 76 natural plants extracts were screened as UV protectants for beet armyworm, *Spodoptera exigua* MNPV (*Se*MNPV). They reported that the mechanism of UV protection of the same virus might be affected by their antioxidant effect as well as UV absorbance (El Salamouny et al. 2009a,b, Shapiro et al. 2009a).

Plant extracts under investigation contain antioxidant compounds such as tannins and flavonoids, which are phenolic compounds. The presence of these polyphenols could play a role in protection from UV light by absorbing the UV itself (Shapiro et al. 2009b). The clove extract is a promising UV protectant, which was also parallel to its UV absorbency when absorption spectra of the extracts were measured by spectrophotometer. This could be because of its content apigenin (Svobodova et al. 2003, Shan et al. 2005). Apigenin present in clove and green tea extracts can protect the skin from cancer caused by UV irradiation effect. Polyphenol (cathecin) presents in the green tea may play role as antioxidant (Shapiro et al. 2009b), while the other additive, henna extract, is not only a natural cosmetic and coloring ingredient but also contains phenolic and flavonoid compounds known for their anti-inflammatory, antifungal, and anticarcinogenic activities (Verghese et al. 2010). Henna also contains tannins that act as a good UV absorber (Musa and Gasmelseed 2012).

The mortality on the olive treatment with no exposure to UV is considerably lower than that on other unexposed treatments. The use of salted olive might cause the pH level of the solution became lower than those other treatments, thus it deleterious to the virus. Findings of this study revealed that clove and henna extracts, with % OAR values observed after 7 d of exposure to sunlight of 79.49 and 66.04%, respectively, were highly effective for virus protection (Table 3). These results suggest that clove and henna extracts might have a potential as UV protectants as compared to kudzu and black tea extracts where the % OAR values were 24.14% and 6.66%, respectively, after 7 d of exposure (Shapiro et al. 2012).

The UV protection in the pomegranate treatment, despite at a moderate rate, could be due to the presence of anthocyanin, tannins, and punicalagin. These substances may act for antioxidant and antitumor activities (Gil et al. 2000). In addition, flavonoid compound in this plant plays a role as antioxidant and antiradical scavenger. Also, it contains vitamin C, vitamin E, and beta-carotene known for their role as UV protectant for skin protection (Zhang and Bjorn 2009).

In the present study, the protection of *Spli*MNP from UV by natural additives could be in part due to the presence of their antioxidant contents and also their ability to absorb the harmful UV light, which prevented the UV light to penetrate and destroy the virus particles from inactivation. As mentioned above, the tested natural additives contain certain phenolic compounds that may play a role in virus protection as UV absorbants (El Salamouny et al. 2009b, Shapiro et al. 2009b).

The results obtained in present study are consistent with those published by Shapiro et al. (2008) where green tea extracts were used as UV protectant for *Spodoptera exigua* (Hübner) (*Se*MNPV). It was concluded that green tea extracts contain several phenol compounds, and the phenolic compound epigallocatechin gallate (Williamson et al. 2006) may play an important role in virus protection (Shepard et al. 2010) in addition to prevention of its DNA damage from UV. Shapiro et al. (2008) have documented that green tea extract is excellent for UV protection of the virus (*Se*MNPV); also, green tea has other important roles such as antibacterial and anticarcinogenic.

The extracts from henna also have antioxidant compounds such as lawsonia and apigenin. Persistence of virus alone treatment (virus

without additives) after 7 d of exposure under the sunny field conditions in this experiment was about 30%. These results are not in agreement with Shapiro et al. (2008) who reported only 3.7% of *Se*MNPV was preserved after the same exposure period. This could be due to the presence of UV protectant additives in the used commercial formulation (Littovir) or there is a possibility that virus concentration used in spray suspension was high which is in agreement with the inactivation inversely related to virus concentration (Shapiro et al. 2012).

The present findings concluded that natural additives clove and henna extracts protected the virus up to 7 d of exposure under sunny conditions. These results are more encouraging than the previous studies (Shapiro et al. 2008, Shapiro et al. 2012), where natural additives kudzu and black tea were used, but *Se*MNPV lost their activity after 2 d of sunlight exposure. The present results clearly indicated that natural additives in virus formulations increase *Spli*MNPV persistence under field conditions.

The *Spli*MNPV application has some advantages, as it is environmentally friendly and relatively has high virulence for several lepidopteran pests such as beet armyworm (*Spodoptera exigua*; Pudjianto et al. 2016) and fall armyworm (*S. frugiperda*; Murillo et al. 2003, El-Sheikh 2015). This study contributed to baculovirus technology for pest management in the field, encouraging further search for effective formulation still effective under the harsh sunny conditions.

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