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Determination of the effect of some plant extracts as the UV-Protectantto the American bollworm, *Helicoverpa armigera*(Hübner)nucleopolyhedrosis virus (*Ha*NPV) virulence under laboratory conditions

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Abstract:*Laboratory studies were initiated to determine the relationship between virus viability and UV exposed time caused inactivation of NPVs from Helicoverpa armigera, the effect of ultraviolet light (UV) protectants on persistence of the entomopathogenic virus HaNPV was also investigated. Asthe exposed times increase the virus sensitivity to UV increases reaching about 90% inactivation after 30-minute, ten plant-derived extracts were tested as ultraviolet (UV) protectants for the nucleopolyhedrovirus (HaNPV). Four of these 10 extracts(black tea, dill, green cabbage and pepper) provided excellent UV protection for HaNPV, these findings indicate that these plant extracts may be useful as UV protectants for the HaNPV and they should beinvestigated further to obtain more efficacious formulations for the control ofagriculturally important insect pests.*

Keywords:*Ultraviolet radiation, Helicoverpa armigera, Plant extracts, UV-protectants, nucleopolyhedrovirus.*

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I. Introduction

Increasing the awareness on the risks of environmental pollution with chemical insecticides and the high demand for producing safe, healthy and pollutant free food leads to searching chemical insecticides alternatives such as microbial-based pesticides. Baculoviruses are considered auspicious control agents against many lepidopterous insect pests as they are host-specific microbes [1]. Baculoviruses (Family: Baculoviridae) are obligate intracellular parasites having either DNA or RNA encapsulated into a protein coat known as capsid to form the virions or nucleocapsids. Baculoviruses are divided into four genera; Alphabaculovirus (Lepidopteran nucleopolyhedrosis virus (NPV)), Betabaculovirus (Lepidopteran granuloviruses (GV)), Gammabaculovirus (HymenopteranNPVs) and Deltabaculovirus (Dipteran NPVs) [2,3].

Viral infection in insects occurs orally, through ingestion of food contaminated with viral inclusion bodies. The replication of the virus in the susceptible tissues of the insect causes their disintegration and the larvae become soft and very fragile [4,5]. According to Alves [6] insects affected by viruses have a lack of appetite, changes in color and lengthening of larval time. In addition, there is a decrease in the activity of the larvae and in the most advanced stages of the infection their death occurs.The infectivity of baculoviruses depends on larval age, however, their multiplication depends on dose, temperature, nutrition, physical characters, and the larval instar [7,8].Presently, many baculoviruses are formulated commercially, however, their application in field is limited because of their susceptibility to inactivation by ultraviolet (UV) in sun-light [7].

However, although these viruses are an efficient tool for the control of pest insects, their field evaluation has presented inconsistent results [5,9], possibly due to the deleterious effect of environmental conditions on viral infectivity[10].Inactivation caused by solar radiation under field conditions is the main environmental factor that limits the massive use of these biocontrol agents [10].Sunlight negatively affects viral particles, particularly radiation in the ultraviolet spectrum and especially radiation between 280 nm and 310 nm [11].The infectivity loss of NPVs and GVs after exposure to direct UV is well documented [8].

The medium-wave or erythemal UV portion of thesun's radiation (UV-B, 280-320 nm) is the most important factor contributing to the photo inactivation of baculoviruses [12].Inactivation occurs also in the near UV region of the solar spectrum (UV-A 320-360 nm), but comparatively greater energydoses are needed as the wave length increases [8,13]. A number of natural UV absorbers provided UV protection to the virusproduct; such as Dyes, Fluorescent brightener or lignin derivates. Antioxidant or oxidative enzyme such as Dilodin, Inol,Vitamins, Folic acid, Riboflavin, and Pyridoxine are all promising compounds for the protection of entomopathogenicviruses from UV-rays [8]. Recently, natural material containing antioxidant such as green tea

[14], Black tea [15], eucalyptus [16] andmango leaf extracts [17] have become promising protective additives to baculoviruses. Therefore, the present study is designed to evaluate ten plant extract as UV-protectants for *Ha*NPV in and determine the most inexpensive, local and natural products to be used as standard protectiveadditives to producean NPV-based product and be applied on field level basis.

II. Materials and Methods

2.1 Insects and virus inoculum:

A colonized strain of the American bollworm, *Helicoverpa armigera* (Hübner) was supplied from the laboratory of Insect Pathogen Unit (IPU), Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC). It was reared for three generations under highly controlled conditions to avoid any insecticides contamination. Larvae were reared on artificial nutrition diet described by [18]. Insects were maintained at 28 ±2°C, 70-80% relative humidity and a 16 h photoperiod[19].

The detection of *Ha*NPV virus was based on external symptom and light microscope examination, and maintained after isolation and propagation at -20° C until requiredas fully described by Ibrahim [29].

2.2 Tested plant extracts:

Ten plant extracts were used as UV-protectant agents and listed in table (1). They were green tea, black tea, pepper, Mango leaf, guava leaf, peppermint, basil, dill, red cabbage and green cabbage. Of each plant, 2 gm was soaked in 100 ml of distilled water and boiled to get the final stock to 2% (w/v), then blended and filtered.

Table (1): Tested plant extracts as UV-Protectants for the American bollworm, *Helicoverpa armigera* nucleopolyhedrosis virus (*Ha*NPV)

Plant	Scientific name	Family
Black tea	*Camellia sinensis* (L.)	Theaceae
Green tea	*Camellia sinensis* (L.)	Theaceae
Pepper	*Capsicum* sp. (L.)	Solanaceae
Green cabbage	*Brassica oleracea* (L.)	Brassicaceae
Red cabbage	*Brassica oleracea* (L.)	Brassicaceae
Mango leaves	*Mangiferaindica* (L.)	Anacardiaceae
Guava leaves	*Psidium guajava* (L.)	Myrtaceae
Basil	*Ocimumbasilicum* (L.)	Lamiaceae
Dill	*Anethumgraveolens* (L.)	Apiaceae
Peppermint	*Mentha × piperita* (L.)	Lamiacae

2.3 UV-irradiation exposure of *Ha*NPV suspension with/without plant extract additives:

2.3.1 Radiation source:

A bactericidal lamp Philips TUV 30 W with a wavelength of 312 nm was used. The UV lamp was vertically placed on a distance far from the prepared viral samples of 160 cm. The irradiation doses were supplied at faculty of agriculture, Ain Shams University, ShobraElkheima district, Qaliobia Governate, Egypt.

2.3.2 Viral suspension concentration:

First, a preliminary bioassay test was carried out to determine the highest viral concentration that causes90-95% larval mortality prior to exposure to UV radiation.The highest determined concentration was 6×10^9occluded bodies (OBs)/ml. This concentration was used as a standard. Then, a set of eight petri dishes were used, each contained 50µl of*HaNPV*suspension of the standard concentration. The viral suspension was spread as thin film on the petri dish surface and left to air-drying. Each petri dish was then exposed to the irradiation source for 5, 10, 15, 30, 60, 120, and 180min, respectively. In addition, a witting agent (2% Triton X) was added to viral suspension (with/without tested plant extracts).

The volume in each petri dish was detected after exposure to UV-irradiation to maintain water loss by evaporation. All petri dishes were then covered by their lids and then stored at 4° C till usage.

2.3.3 Effect of UV-irradiation with plant extracts addition:

The effect of UV-irradiation with plant extract addition to viral suspension was evaluated according to the prescribed method by Shapiro *et al*. [14]. The plant extract was added to prepared virus inoculum and the final concentration was adjusted 1% (including the standard viral concentration). As previously mentioned in section 2.3.2, the petri dishes with viral suspension and plant extract additive was prepared and exposed to the UV-radiation source for 30 min. After the exposure time, the volume was determined and distill water was added to each dish to replace the lost water by evaporation.

2.4 Bioassay test:

In order to evaluate the effect of UV-irradiation effect on *Ha*NPV virulence, a laboratory bioassay was performed by diet surface contamination technique.When UV-treated dishes were removed from refrigerator, 50 µl of virus suspension (either *Ha*NPV/water or *Ha*NPV/tested plant extract) was applied to the diet surface

each30-ml plastic cup. In addition, the nonirradiated *Ha*NPVin water also was utilized into cups as a positive control.Thirty3rdinstar larvae of *H. armigera* were starved for 10h and placed in each cup and incubated at 30° C under laboratory conditions. Tests were repeated three times with ten larvae per treatment, ten untreated larvae and ten test treatments controls per replicates.

Cumulative larval mortality was recorded on daily basis starting on the 5th day post treatment and continued to 14th day as the experiment was ended.The percentage of original activity remaining(% OAR) was used as the basis of UV protectionand was based upon virus-caused mortalitybefore and after radiation [10,20].

$$\% \text{ OAR} = \frac{M_T}{M_c} \times 100$$

Where M_T is the treated larval mortality and M_C is the control larval mortality [30].

2.5 Statistical analysis:

Larval mortality of treated larvae was corrected according to Abbott's formula [21]. Mean were tested for significance by using the one-way analysis of variance (ANOVA) using SPSS statistics 17.0 release 17.0.0 software [22].

III. Results and Discussion:

3.1 Crude preparation of *Ha*NPV from virus killed insects:

The present study was designated to initiate a basic data for further studies for the effect of UV-irradiation on the entomopathogenic virus and the suitable, low cost, and safe UV-protectant. In the present investigation, crude aqueous suspensions of *Ha*NPVwere obtained from virus killed insects. Previously researches used either crude, technical products, or purified products for inactivation studies [23]. Although crude preparations from virus killed insects have greater biological activity than purified preparations [24] but homogenized host tissues and pigmentation can affect UV absorbance and consequently influence the UV-radiation results [25].

3.2 Effect of UV-irradiation on the virus infectivity:

Data presented in table (2) showed the effect of UV-irradiation on viral infectivity according to duration of exposure. Accumulative larval mortality was recorded during 14days post treatment. Results showed that the isolated virus had a good infectivity as the recorded larval mortality of larvae treated with non-radiated viral preparation was 93.3% and OAR percentage of 100%. Results also showed that as the UV-radiation exposure duration increased the larval mortality greatly decreased as an indication of viral infectivity loss. In addition, results showed that 90% of viral infectivity was lost after 30 min. of exposure. Complete viral inactivation was obtained after 60 min. of irradiation as no larval mortality was recorded.

Table (2): Pathogenicity of *Ha*NPV against 3rd instar larvae of *H. armigera* after exposure to UV-radiation for different time

Exposure time (min.)	Mean larval mortality (± S.D)	% Mortality	% OAR
0	28.0 ±0.33	93.3	100.0
5	24.0 ±0.00	80.0	85.7
10	23.0 ±0.33	76.0	82.1
15	5.0 ±0.33	23.3	25.0
30	2.0 ±0.33	6.6	7.1
60	1.0 ±0.33	3.3	3.5
120	0.0±0.00	0.0	0.0
180	0.0±0.00	0.0	0.0

This was concurrent with that obtained by El-Salamony*et al*. [1] and Shapiro *et al*. [26] who indicated that *SeMNPV* should be exposed to UVA/UVB radiation for 30 min to reduce virus activity below 10%. Moreover, results obtained by Shapiro *et al*. [14] which reported that 98.7% of *Spli*NPV pathogenicity after exposure to UV-radiation.

3.3 Viral infectivity of *Ha*NPV in presence of additives:

The effectiveness of ten plant extracts as UV-protectant was tested as added to irradiated viral suspension and furtherly applied to the 3rd instar larvae of the American bollworm (Table 3).According to obtained data in section 3.2., all plant additives were exposed to UV-irradiation source for 30 min. Results showed that black tea, pepper, green cabbage, and dill extracts provide good UV-protectant for the virus. This was obvious from larval mortality as it ranged from 90-83.3% and OAR percentage values ranged from 96.4-89.2%. On the other hand, basil, mango and guava leaf extract provide the lowest UV-protection as they showed the lowest larval mortality and lowest OAR percentage values.

Table (3): Pathogenicity of *Ha*NPVagainst 3rd instar larvae of *H. armigera* after addition of plant extracts and exposure to UV-radiation for 30 min.

Exposure time (min.)	Mean larval mortality (± S.D)	% Mortality	% OAR
Virus alone	2.0 ± 0.33	6.6	7.1
Black tea	27.0 ± 0.33	90.0	96.4
Green tea	17.0 ± 0.21	56.6	60.7
Pepper	25.0 ± 0.00	83.3	89.2
Green cabbage	25.0 ± 0.33	83.3	89.2
Red cabbage	24.0 ± 0.12	80.0	85.7
Mango leaves	14.0 ± 0.33	46.6	50.0
Guava leaves	16.0 ± 0.00	53.3	57.1
Basil	21.0 ± 0.12	70.0	75.0
Dill	26.0 ± 0.33	86.6	92.8
Peppermint	23.0 ± 0.00	76.6	82.1

In addition, results showed that both green and black tea acts as UV protectants but black tea has the most protectant effect. This might be due the presence antioxidants in tea, as well as the vegetables such as cabbages, dill and peppermint that give high protectant effect and known to be rich antioxidant compound and polyphenols, which scavenge free radicals and reactive oxygen species [27]. Shapiro *et al*. [28] utilized plant extract as UV-protectant for entomopathogenic viruses. They tested green, black tea and coffee as UV-protectants to beet armyworm nucleopolyhedrosis virus.

In summary, we feel that we have optimized the laboratory screening systemfor further laboratory and field tests. The immediate goal of this study was to assess the effectiveness of 10 plant derived extracts as UV protectants for the beet armyworm NPV (*Ha*NPV) as part of our program on non-chemical control of insect vegetable pests. In this regard, our goal was achieved as we identified four plant extracts (black tea, dill, green cabbage and pepper) that provided respectively a highest level of protection followed by the red cabbage and peppermint and will be tested in future field experiments. Moreover, it is hoped that this research may lead to further interest in the use of plants and plant products as adjuvants for insect pathogenic viruses in pest management of agriculturally important insects.

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