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Effect of Ultraviolet Radiation on Original Activity Remaining of *Spodoptera littoralis* NPV against *S. littoralis* Boisd (Lepidoptera: Noctuidae)

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THE COTTON leafworm, *Spodoptera littoralis* (Boisd) is a serious pest of cotton and other important plants in Egypt. The use of chemical insecticides caused environmental pollution. So that there is strong need to find other safer method than chemical pesticides for insect pest control such as entomopathogenic baculoviruses. The UV effect on *Spodoptera littoralis* Nuclear Polyhedrosis Virus was studied. The results indicated that exposure of the stock virus concentration (1.1 x 10^8 Polyhedral Inclusion Bodies per milliliter (PIB /ml)) to Ultraviolet (UV) for 5.0 and 20.0 minutes lead to decrease in the number of PIB/ml, where the PIB/ ml became 2.9x 10^6 and 6.125 x 10^5, respectively . These concentrations caused 50% larval mortality after 18.72 and 21.88 days respectively, while the original non exposed concentration (1.1 x 10^8) caused 50 % larval mortality after 4.29 days. The results indicated that UV exposure decreased virus concentration, efficacy of virus and Original Activity Remaining. The irradiated virus activity decreased and the corresponding percentage of original activity remaining decreased. The results confirmed that applying the virus most is before sunrise and /or sunset. Also, it is necessary to use natural additives to protect the virus from UV radiation.

Keywords: *Spodoptera littoralis*, Chemical insecticides, Entomopathogenic virus, Ultraviolet radiation (UV), Polyhedral Inclusion Body (PIB), Nuclear Polyhedrosis Virus, Original Activity Remaining.

Introduction

Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) is one of the most destructive polyphagous insects against field crops. It belongs to arthropod (mainly mite and insects) have many economically important pests. [1, 2].

The chemical insecticides caused toxicity to humans, domestic animals and beneficial insects. Also, resistant insect development to traditional pesticides [3].

It needs to find alternative pesticides for insect pest control such as entomopathogenic baculoviruses.

The Nuclear Polyhedrosis Virus (NPV) is a double stranded DNA baculovirus being highly specific on their host insects. So that they are safe to humans, the environment, natural enemies and plants [4-6]. DNA is a polymer of deoxyribonucleoside monophosphates covalently linked by 3'--› 5' phosphodiester bonds. DNA exists as a double-stranded molecule in which the two strands wind around each other, forming a double helix stabilized by H-bonding between bases in adjacent strands [7-8]. Solar inactivation has been a main inhibition for insect pathogenic viruses [9-11] and is moderately responsible for inconsistent field efficiency. In many cases, viral

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activity is significantly decreased within 24-48 h. [12- 13].

In field applied pathogens lose at least 50% of their original activity within several days [10] and in other cases within 24 h [14]. Jones and McKinley [7] demonstrated that more than 90% inactivation of *Spodopteralittoralis* Multinucleocapsid Nucleopolyhedrovirus (*Sl*MNPV) happened within 4 h and that more than 99% inactivation occurred within 8 h, under natural conditions. They concluded that almost all of the inactivation was due to UV-B (i.e., 305-320 nm).

All of mentioned above confirmed that the biosafety and specificity of Ultraviolet non exposed Nuclear Polyhedrosis Virus recommend using of this virus as an alternative method to chemical insecticides in Integrated Pest Management Program.

This work is designed to study the effect of UV radiation on the effectiveness of the cotton leaf worm, *S. littoralis* NPV.

Experimental

Insect rearing

A laboratory colony of the Egyptian cotton leafworm, *S. littoralis*, was used in the study. The insect originated from the laboratory of Pests and Plant Protection Department, National Research Centre, Egypt. Larvae of *S. littoralis* were reared on caster leaves (*Ricinus communis*) in the laboratory for a year (~10 generations) away from any insecticide exposure at 27.0 ± 2.0 ºC and 65.0 ± 5.0% Relative Humidity (RH) with a photoperiod of 16:8 h (light:dark).

Virus isolation and production

The original virus was produced and isolated from diseased larvae of *S. littoralis* by Samah M.M.A. according to [2] at Pests & Plant Protection Department laboratory, National Research Centre, Egypt.

Preparation of Nuclear Polyhedrosis Virus concentrations for exposure to UV.

The original stock, 1.1 x 10^8 was counted under light microscope by hemocytometer according to [16].

Two concentrations of 2.9x10^6 and 6.125x10^5 PIB/ml were resulted from 1.1 x 10^8 (the original stock concentration) after exposed to UV for 5.0 and 20.0 minutes, respectively.

The concentrations of non-exposed or exposed samples were maintained at -20.0 Cº till use.

Evaluation of the insecticidal activity of Nuclear Polyhedrosis Virus concentrations.

A preliminary experiment was carried out to estimate the lethal concentrations and the lethal times of UV non-exposed and UV exposed Nuclear Polyhedrosis Virus concentrations against newly molted *S. littoralis* fourth larval instar until final larval stage.

Twenty newly molted *S. littoralis* fourth larval instars were placed in plastic cups (8 cm diameter and 5 cm height) for each replicate and left to fed on castor leaf disks (5 mm diameter). Each disk was treated using a Handle sprayer (20.0 ml capacity) with the prepared viral concentrations containing Occlusion Bodies (OBs) (non-exposed and exposed to UV). Control larvae were fed on castor disks (*Ricinus communis*) treated with distilled water without virus. After two days, the treated and untreated castor disks were removed and replaced by another fresh untreated castor leaf disks. The experiment was continued till the final larval stage (5^{th} larval instar) or death of larvae. Three replicates were used for each bioassay for each treatment and for control as described above. Larval mortality was recorded daily. The method of percentage mortality calculations was described in [17] and the Probite analysis (Lethal concentration (LC) & lethal time (LT) was described in [18].

$$\text{Corrected mortality \%} = \frac{T - C}{100 - C} \times 100$$

Where: T = larval mortality in treatment and C = larval mortality in control

Original Activity Remaining (OAR) was calculated according to [19]

$$\text{OAR} = \frac{\text{\% larval mortality after exposure}}{\text{\% larval mortality before exposure}} \times 100$$

The percentage of original activity remaining was based upon virus-caused mortality before and after irradiation at the same virus concentration for each exposure period for each treatment.

Statistical analysis

The statistical analysis was carried out by program of SPSS version (19) using:

1. General Linear Model Univariate Anova Test (Two ways Anova test)
2. Correlate Bivariate.

Results and Discussion

The results showed that exposure of the stock virus (1.1 x 10^8) to UV for 5.0 and 20.0 minutes lead to decrease in the number of PIB/ml, where the PIB/ ml became 2.9x 10^6 and 6.125 x 10^5, respectively. These concentrations caused 50 % mortality for larvae after 18.72 and 21.88 days respectively, while the original non exposed concentration (1.1 x 10^8) caused 50 % mortality of larvae after 4.29 days. These concentrations (2.9x 10^6 and 6.125 x 10^5) caused 35.18 and 24.07 % mortality of larvae during twelve days but the original non exposed concentration (1.1 x 10^8) caused 79.96% mortality of larvae during twelve days. Results confirmed that the efficacy of exposed virus to UV became very weak. Also, whenever UV exposure period increased the concentration of virus decreased and larval mortality decreased (Table 1). The percentage of Original Activity Remaining (OAR%) of virus after exposed to UV for 0.0, 5.0 and 20.0 minutes was calculated to record 100%, 43.9% and 30.1%, respectively. This means that exposed virus to Ultraviolet rays lose about 56.1% and 69.9% of its OAR%, respectively. El-Helaly et al.,[20] found that the recorded rates of mortality among *S. littoralis* neonate larvae caused by *Spodoptera littoralis* Nuclear Polyhedrosis Virus (*Spli* NPV) virus after exposure of *Spli* NPV virus for 10, 24, 48, 96 and 168 hours to natural conditions were 96.00%, 48.78%, 6.97%, 0%, and 0%, respectively, compared to 100.00% in case of un-irradiated virus (the calculated LT_{50} was only 24.07 hours(h). Also, the authors., [20] found that there was not any OAR% remained in examined additives expect of cacao after 168 h which gave 13.15 OAR% for the concentration of 5%, for 10% concentration only cacao and green cabbage have 17.94 and 2.5 OAR%, respectively, after 168 hours. Elnagar and Abul-Nasr [21] found that under sunlight UV purified virus suspension was less effective than the crude extract as the latter contains coloring material. In Egypt, the effect of sunlight on *Spli*MNPV was thoroughly investigated [22,23].

Griego et al., [24] found that the inactivation of Multinucleocapsid *O. pseudotsugata* Nuclear Polyhedrosis Virus by monochromatic UV light decreased mortality rate in the groups fed irradiated virus compared with those fed non irradiated virus. The irradiated virus activity decreased and the corresponding percentage of original activity remaining decreased. Also, the authors.,[24] showed that virus inactivation increased with increase in flounce, regardless of the wavelength used , As wavelength was increased, however, the flounce had to be increased to cause the same degree of inactivation caused by the shorter wavelengths, The Spearman rank correlation coefficient test showed a reverse relationship between flounce and percentage of original activity remaining at each wavelength ($P < 0.05$), indicating that as the dose was increased the degree of virus inactivation was also increased , At a flounce of 1.152 x10^3 w/m^2, the percentages of activity remaining after exposure to wavelengths of 290, 300, 310, and 320 nm were 15.4,28.2, 41.0, and 71.8, respectively. Effect of exposure periods to UV on virus was studied previously. Akhanaev et al., [25] studied the open area sunlight exposed virus strains for 0.25, 0.5, 1, and 2 hours and later per orally inoculated host larvae with the same doses of virus (5x10^5) and with doses leading to same effect ((Lethal Dose killing 90% of the insect pest (LD90)). Also, they observed that strain *LdM* NPV-45/ *O*, which previously gave high virulence against *L. dispar* larvae, was more sensitive to UV irradiation. Sunlight exposure caused a significant delay of *LdM*NPV-45/0-induced pathogenesis already after 0.25 h of sunlight exposure, while for *LdM*NPV-27/0, the delay was occurred only after 2 h exposure in spite of used concentrations. Also, the authors., [25] compared the sequences of the main structural proteins of the studied strains as UV light contributes not only to genome damage in viruses but also to structural protein damage.

Prabhu and Mahalingam [26] studied the inactivation effect of sunlight and UV light on the Nuclear Polyhedrosis Virus to

TABLE 1. Effect of exposure periods to UV on *S. littoralis* NPV.

Exposure period ToUV (minute)	Concentrations result **(PIB/ml)	Mortality%	LC_{50}	LT_{50}	*OAR%
0.0	1.1x10^8	79.96		4.29	100
5.0	2.9x 10^6	35.18	7.7 x10^6	18.72	43.997
20.0	6.125x10^5	24.07		21.88	30.1

*OAR% (Percentage of Original Activity Remaining)

**PIB/ml (Polyhedral Inclusion Bodies per milliliter)

Diaphania pulverulentalis (Hampson) larvae under laboratory conditions and observed that Significant difference in larval mortality when *Dp*NPV was exposed to 5, 10, 15, 30 and 60 minutes to UV light, (88.33, 85.00, 80.00, 66.66 and 53.33 % mortality, respectively. Whereas, in formulated *Dp*NPV, the rate of larval mortality recorded was higher (93.33, 90.00, 86.65, 71.66 and 66.66 % respectively). The inactivation of virus was directly related to the period of exposure to UV radiation, the viral activity of the irradiated suspensions decreased with increased exposure duration to UV light.

The F value (resulted from F statistical test in two way Anova test shows if a group of variables are jointly significant) between the UV exposed and UV non exposed virus per day was significant. Virus persistence was calculated as OAR% (Percentage of Original Activity Remaining) based upon 100% at no UV exposure period. The OAR% for (the resulted concentrations, $2.9x10^6$ and $6.125x10^5$ from 5 and 20 minutes UV exposed virus) were 43.9% and 30.1% respectively. The results were analyzed by SPSS Program and showed that significant differences were found between the UV exposed and UV non exposed virus with the exception of 5 min UV exposed *Spodoptera littoralis* Nuclear Polyhedrosis Virus (R1.1) & 20 min UV exposed *Spodoptera littoralis* Nuclear Polyhedrosis Virus (R1.2) probability value ((P value) was 0.222 more than 0.05) and R1.1& Control (R) (P value was 0.094 more than 0.05) where no significant differences were found.

The correlation between concentrations, time of UV exposure, corrected mortality and OAR% were significant. From these data there was strong and significant reversible correlation between each of (time of UV exposure & concentration), and between (time of UV exposure & corrected mortality), (time of UV exposure & OAR%) because the value of correlation factor is (-) and near to 1 (-0.801, -0.848, -0.848 respectively) and has very high significance (P value was 0.0 Less than 0.05).

There was strong and significant irreversible correlation between each of the following (concentration & mortality), (concentration & OAR), (OAR & corrected mortality) because the value of correlation factor is (+) and near to 1 (0.997,0.997,1 respectively) and has very high significance value (P value was 0.0 less than 0.05). (Table 2).

In the nearly future, the use of entomopathogenic virus might be one of the integrated pest management.

TABLE 2. Statistical Correlation between Time of UV exposure, concentration, corrected mortality and OAR% by using Pearson Correlation.

		Concentration	Time of UV exposure	Corrected mortality	OAR%
Concentration	Pearson Correlation	1	-.801	.997	.997
	Sig. (2-tailed)	-----	.000	.000	.000
	N	113512500	113512500	113512500	113512500
T	Pearson Correlation	-.801	1	-.848	-.848
	Sig. (2-tailed)	.000	-----	.000	.000
	N	113512500	113512500	113512500	113512500
Corrected mortality	Pearson Correlation	.997	-.848	1	1.000
	Sig. (2-tailed)	.000	.000	-----	.000
	N	113512500	113512500	113512500	113512500
OAR%	Pearson Correlation	.997	-.848	1.000	1
	Sig. (2-tailed)	.000	.000	.000	-----
	N	113512500	113512500	113512500	113512500

Conclusion

Theoriginalconcentration ($1.1x10^8$) was the most efficiency followed by $2.9x\ 10^6$ and $6.125x10^5$ being the least efficacy. Results recommend that there is a need to applying the entomopathogenic virus in the suitable time before sunrise and sunset to avoid the natural radiation which decreases the virus efficacy. It is also necessary to add materials for protecting the virus from the UV rays.

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تأثير الاشعة الفوق بنفسجية على النشاط الاصلى المتبقى للفيروس البوليهيدرى النووى المعزول من دودة ورق القطن (Spodoptera littoralis NPV) ضد دودة ورق القطن (Lepidoptera: Noctuidae) *S. littoralis* (Boisd)

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تعتبر دودة ورق القطن *Spodoptera littoralis* آفة خطيرة على القطن والنباتات الهامة الأخرى في مصر. ان استخدام المبيدات الحشرية الكيميائية تسبب في حدوث التلوث البيئي. لذا أصبح هناك حاجة قوية للعثور على طريقة أخرى أكثر أمانا مثل الفيروسات الممرضة للحشرات. تمت دراسة تأثير الأشعة فوق البنفسجية على الفيروس المعزول من يرقات دوده ورق القطن. أشارت النتائج إلى أن تعريض التركيز الأصلي من الفيروس (١,١ × ١٠^٨) للأشعة فوق البنفسجية لمدة ٥,٠ و ٢٠,٠ دقيقة ادى إلى انخفاض في عدد PIB / مل، حيث أصبح PIB / مل ٢,٩ × ١٠^٦ و٦,١٢٥ × ١٠^٥، على التوالي. وهذه التركيزات حققت ٥٠ ٪ موت لليرقات بعد ١٨,٧٢ و٢١,٨٨ يوما على التوالي، في حين أن التركيز الأصـــلي غير المعــرض (١,١ × ١٠^٨) حقق ٥٠ ٪ موت لليرقات بعد ٤,٢٩ يوما. وأظهرت النتائج أن تعرض الفيروس الممرض للحشرة للأشعة فوق البنفسجية قلل من تركيزه وفعالية النشاط الأصلي الباقي للفيروس. كما اتضح ذلك من انخفاض في النسبة المئوية للنشاط الأصلي المتبقي للفيروس. أكدت النتائج أن تطبيق الفيروس الممرض للحشرات لابد ان يطبق قبل شروق الشمس و / أو غروب الشمس. أيضا، فمن الضروري استخدام الإضافات الطبيعية لحماية الفيروس الممرض للحشرات من الأشعة فوق البنفسجية.