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THE IMPACT OF ULTRAVIOLET RADIATIONS ON THE MULTINUCLEOCAPSID NUCLEAR POLYHEDROSIS VIRUS' INSECTICIDAL ACTION AGAINST *AGROTIS IPSILON* (H.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

In its hot and humid areas, the black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is one of the most harmful and destructive polyphagous agricultural pests of various field crops. It can attack a variety of commercially significant crops and consistently causes significant feeding harm to cotton, vegetables, and some ornamental crops in Africa, Asia, and Europe. The impact of ultraviolet light on the insecticidal activity of *Spodoptera littoralis* Multinucleocapsid Nuclear Polyhedrosis Virus against the black cutworm *Agrotis ipsilon* (Hufnagel) was examined in laboratory experiments. (Lepidoptera: Noctuidae).

From the initial stock, three diluted concentrations were taken, and the haemocytometer counted the number of Polyhedral Inclusion Bodies (PIB)/ml/concentration under a light microscope. The PIB numbers were $1.1x10^8$, $3.11x\ 10^7$, and $6.7x\ 10^6$ for each concentration (1.0 ml), correspondingly. The percentage mortalities to 4th larval instar larvae tested until mortality or pupation for each of these concentrations were 79.96, 78.84, and 61.44%, respectively. Three groups were created for each percentage. The first group did not receive any UV exposure, while the second and third groups received 5.0 and 20.0 minutes of UV

exposure, respectively. The PIB/ml were measured and tested against fourth instar larvae fed on castor leaf discs treated with aqueous suspensions of nuclear polyhedrosis virus after the exposure interval. According to the calculations, the LC50 values for the concentrations before and after UV radiation were 1.3 x 10^6, 7.7 x 10^6, 2.97 x 10^6, and 6.1 x 10^6 PIB/ml, respectively. Lethal times (LT50s) for each concentration were also measured, and the results showed that the lethal time reduced as virus concentration increased.

Additionally, it was discovered that lengthening UV exposure time was followed by a decline in virus concentration and mortality rate.

Keywords: Ultra Violet, insecticidal activity, Nuclear poly hedrosis virus, *Agrotis ipsilon*

Background

In its hot and humid areas, the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is one of the most harmful and destructive polyphagous agricultural pests of various field products (Carter, 1984). In Africa, Asia, and Europe throughout the year, it can attack a variety of economically significant crops and produces significant feeding damage to cotton, vegetables, and some ornamental crops (Bayoumi et al., 1998). More than 40 insecticide formulations from various organisations have been registered and used to control this insect pest as it severely damages many crops in Egypt in accordance with the approved agricultural pest control guidelines. (Abd El-Salam& ElHawary,2009; Anonymous, 2012). Widespectrum insecticides have been used extensively in the last few decades to combat *A. ipsilon*, which has resulted in the evolution of resistance to many insecticides that have been registered (Arivudainambi, S., 2000). Conventional pesticides, particularly those containing organophosphorus and pyrethroid insecticides, are typically used to control agricultural insect pests, but their indiscriminate use may result in environmental pollution, toxic residual effects, the development of pest resistance, negative effects on non-target organisms, and negative effects on human and animal health. (Sundararaj, 1997). Due to the fact that plant-derived molecules are environmentally friendly, biodegradable, and target-specific, and because there has not yet been any documented evidence of pests developing resistance to them, these issues compelled

scientists to look for novel control measures, particularly those from plant origins (Gautam et al, 2013). Currently, nucleo polyhedro virus (NPV) (Baculoviridae) is a naturally occurring microbial pathogen of Lepidoptera that is extremely specific to its host insects and safe for people, other animals, plants, and natural enemies (Yang et al., 2012). It is an ideal tool in pest management programmes. However, some of the issues that have restricted its use are the confined host range, the slow speed of kill, the high dose requirement, and the emergence of resistance. These issues have created new opportunities for NPV and its formulas to be improved (Abdel-Hamid et al. 1996, Moscardi 1999, Dalia et al. 2009, El-Menofy et al. 2014). In this situation, adding adjuvants like neem seed preparations to NPV had improved its effects (Kumar et al., 2008; Bajwa and Ahmad, 2012; Wakil et al., 2012; Zamora-Avilés et al., 2013). Therefore, it is necessary to assess the species-specific NPV efficacy.

Determining how Ultra Violet affects the virulence of Multinucleocapsid Nuclear Polyhedrosis Virus (Spli.NPV) and the mortality caused by the virus to *A. ipsilon* (fourth larval instar to final larval stage) as well as the yield of this viral isolate from *Spodoptera littoralis* is the goal of the current study. The information could be useful in developing a pest management plan

METHODS

Insect rearing

Twenty newly emerged *A. ipsilon* moths were acquired from the cutworm department, moved to the laboratory of the Vegetables Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, and kept in glass jars covered with tissue and held in place by rubber bands. Adults consumed a 20% concentration of honey solution as nutrition. Females were permitted to deposit their eggs on muslin strips fixed to the jars' tops. After egg laying, these strips were put into Petri dishes and maintained in an incubator at a constant temperature of 25 °C 1 °C and 70–80 RH% until the eggs hatched (Zhang et al. 2019). Castor leaves were added daily to the recently hatched larvae's small glass jars as a source of food. To prevent cannibalism, the 4th-instar larvae were placed in a glass plate either singly or in small groups.

Virus propagation

The original virus isolate was found in the El-Beheira Governorate, where infected *S. littoralis* larvae were physically collected from cotton, tomato, and maize fields. The larvae that showed signs of baculovirus infection were brought to our lab. Individually maintained larvae were housed at -20 °C in 15 ml plastic tubes. Larvae were individually homogenised in ddH_2O and centrifuged at 100 g for 3-5 min. to remove cell debris in order to prepare them for an initial phase-contrast microscopic analysis and for viral propagation. To aid in the precipitation of OBs, the supernatant was then centrifuged at 5000 g for 10 min. The pelleted OBs were re-dissolved in an appropriate volume of ddH_2O. Under a phase-contrast microscope, a small number of OBs were analysed. Purifed OBs for each isolate were used to inoculate about fifty fourth instar larvae by feeding them a small piece of medium contaminated with few microliters of each isolate for 24 h. Next day, larvae that ingested the viral-contaminated medium were transferred to a fresh medium. The dead larvae were collected a few days after infection (p.i) and submitted to OBs purification while the infected larvae were kept in regulated ambient settings.

Virus occlusion bodies' counting

Purification of viral OBs was done in accordance with Boughton et al. (1999). *S. littoralis* larvae that were infected were collected and homogenised with 0.1% SDS, and then filtered through cotton and filter paper. The suspension was centrifuged, and the pellets were then re-suspended in 0.5 M NaCl and the final pellet, which included OBs, was re-suspended in an appropriate volume of ddH_2O. Using an inverted microscope (Axio-VertA1, Zeiss, Germany) and a dark field, the diluted OBs were counted. Petroff-Hauser counting chamber (depth 0.01 mm, Hausser Scientific) was used for the counting. The purified viral OBs have been examined using both a light and an electron microscope after being frozen at -20°C.

Electron microscope Examination

The purified OB suspensions were put onto carbon-coated grib, stained with 2% phosphor tangistic acid, and then examined under a transmission electron microscope TEM (JEOL model JEM-1200EX II).

Purification of viral DNA

The purified viral OBs were used to isolate the SpliNPV genomic DNA using the technique described by Boughton et al. (1999). In brief, 1M of Na2CO3 was used to dissolve the purified OBs and release virions (ODV). ODV were then treated with 10% (w/w) SDS before being given a further 1%. Genomic DNA can be released by using proteinase K. By washing the cell debris two times with TE-buffer saturated phenol/chloroform 1:1 (v/v), the nucleic acids were removed from the debris. Using a 1:10 volume of 3M NaAc pH 5.2 and 96% ethanol, the viral genomic DNA was precipitated. The cleaned genomic DNA was then eluted in an appropriate volume of ddH_2O after being washed with 70% ethanol.

Viral DNA digestion

The study was done by comparing the number of reference strain fragments to the number of common fragments of the tested isolates produced SpliNPV-EL-Beheria by restriction endonucleases (PstI). The findings of the digestion employing PstI endonuclease on the SpliNPV-EL-Beheria no variations in their patterns were found (Fig. 2).

PCR amplifcation and sequencing of polyhedrin gene

According to the procedure previously described by Elmenofy et al. (2020), one pair of specific primers termed Spli-polh-F (5-ATCTGGGCAAAACCTATGTAT-3) and Spli-polh-R (CTTGGCGAGACTGATGCGGTATTC) were designed, synthesised, and utilised for amplifcation of the polyhedrin gene fragment. In a total volume of 25µl, the following ingredients were used in the PCR reactions: 2µl of viral genomic DNA template (500ng), 12.5µl of Takara Bio's EmeraldAmp® GT PCR Master Mix, and 1 µl of each forward and reverse primer. Using autoclaved ddH_2O, the volume was finished to a capacity of 25µl. The PCR programme started at 95°C for 3 min, then went through 35 cycles of primer extension at 72°C for 45 s, primer

denaturation at 95°C for 1 min, and primer extension at 60°C for 1 min. The primer extension was finished after 7 minutes at 72 °C. A 1% agarose gel produced in 1X TAE buffer was used to electrophorese the PCR-amplicon. Using a UV transilluminator, the PCR amplicon was seen. The Qiaquick PCR purification kit (Qiagene, Germany) was used to separate the PCR fragments from the agarose gel. Sanger sequencing was used to put the purified fragments in order.

Phylogenetic analysis

Using the National Centre for Biotechnology Information's (NCBI) Blast search database, the SpliNPV-EL-Beheria sequence was subjected to comparison with published ones. Using the sequence analysis programme Windows 32 Edit Seq 4.00 (1989-1999) from EditSeq-DNAstar Inc., the inferred amino acid sequence was examined. The phylogenetic tree and multiple sequence alignment studies were completed using Clustal Omega (The EMBL-EBI search and sequence analysis tools APIs in 2019).

Exposure of the virus OBs to UV-irradiation

A two-group well plate containing the virus was exposed to UV light using Philips TUV 15W G5 T8 germicidal bulbs at a wavelength of 254 nm for 5 minutes for the first group and 20 minutes for the second group, respectively.

Nuclear polyhedrosis virus concentrations are prepared for UV radiation

From the initial stock, three diluted concentrations were taken, and the haemocytometer counted the number of PIB/ml/concentration under a light microscope. The PIB numbers were $1.1x10^8$, $3.11x\ 10^7$, and $6.7x\ 10^6$ for each concentration (1.0 ml), correspondingly. Each concentration was kept at -20.0 Co in deep freezing. After being removed from the deep freezer, each dose was brought to room temperature (25.0± 2.0 $C^{o)}$.

We divided each concentration into three categories. The first group did not receive any UV exposure, while the second and third groups received 5.0 and 20.0 minutes of UV exposure, respectively. The PIB/ml was measured following the exposure time. The concentration of PIB/ml in

the first group, which was not exposed to UV light, was ($1.1x10^8$), the concentration in the second group was ($2.9x10^6$), and the concentration in the third group was ($6.125x10^5$). The PIB/ml values for the second concentration, $3.11x10^7$ PIB/ml (non-exposed), decreased to $2.9x\ 10^6$ and $9.4x10^5$, respectively, after being exposed to UV for 5.0 and 20.0 minutes. Additionally, after being subjected to UV for 5.0 and 20.0 minutes, the third concentration, which was $6.7\ x\ 10^6$ PIB/ml (nonexposed), changed to $4.1\ x\ 10^6$ and $1.5\ x\ 10^6$ PIB/ml, respectively. The amounts of the exposed and unexposed samples were then kept constant at -20.0 C^o until use.

Assessment of nuclear polyhedrosis virus amounts' insecticidal effects

Using recently moulted *A. ipsilon* fourth larval instar larvae, a preliminary experiment was conducted to determine the lethal concentrations and the lethal times of UV non-exposed and UV exposed Nuclear polyhedrosis virus.

For each replicate, twenty newly moulted fourth larval instars of the *A. ipsilon* species were placed in plastic cups (8 cm in diameter and 5 cm in height) and allowed to feed on castor leaf discs (5 mm diameter). Using a Handle sprayer (20.0 ml volume), each disc was treated with the prepared viral concentrations containing OBs (non-exposed and exposed to UV). Control larvae were fed on castor disks (*Ricinus communis*) treated with distilled water without virus. The treated and untreated castor discs were removed after two days and substituted with brand-new, untreated castor leaf discs. The experiment was continued till the final larval stage or death of larvae. For each bioassay for each treatment and for the control, three duplicates with 60 larvae were used. Daily records of larval mortality were kept, Abbot's (1955) formula was used to determine percentage mortality, and Finny's (1971) Probite Analysis was performed.

Statistical analysis

Based on Finney's (1971) description of the probit analysis technique, lethal concentrations (LC) and lethal times (LT) were calculated.

RESULTS

Examining SpliNPV OBs using an electronic microscope

Typical viral infection symptoms were found 5-7 days after infection, and pure viral OBs indicated that the isolated virus was special to *S. littoralis* larvae. The transmission electron microscope (TEM) and light microscope were used to study the viral OBs. visible polyhedra were visible in all surfaces upon microscopic analysis. Additionally, transmission electron microscopic examination of the viral OBs revealed that they are typical baculovirus OBs of the nucleopolyhedrovirus (NPV) type with polyhedral structures (Fig. 1).

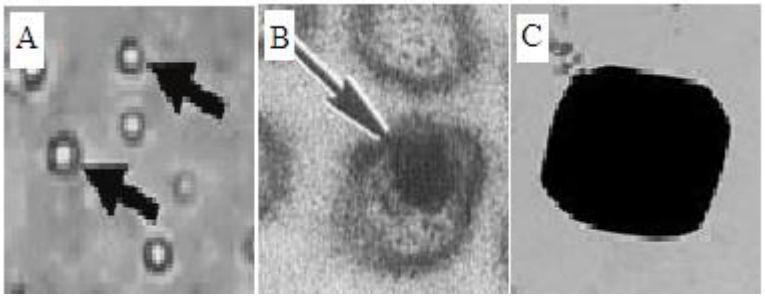

Fig. 1 OBs of *Spodoptera littoralis* SpliNPV captured in electron micrographs. **a** SpliNPV occlusion bodies as seen under a light microscope. **b** Transmission electron micrograph with the red arrow designating the SpliNPV occlusion bodies. **c** A viral occlusion body with a polyhedral shape that is typical of NPV. Bar = 100 nm.

Restriction profle analysis

To examine the restriction patterns produced by the three SpliNPV isolates, the genomic DNA of each was digested with the enzyme PstI (Fig. 2). Particularly for digestion with PstI, which showed no variations in their patterns of SpliNPV-ElBeharia were found compare to the reference strain SpliNPV-AN1956.

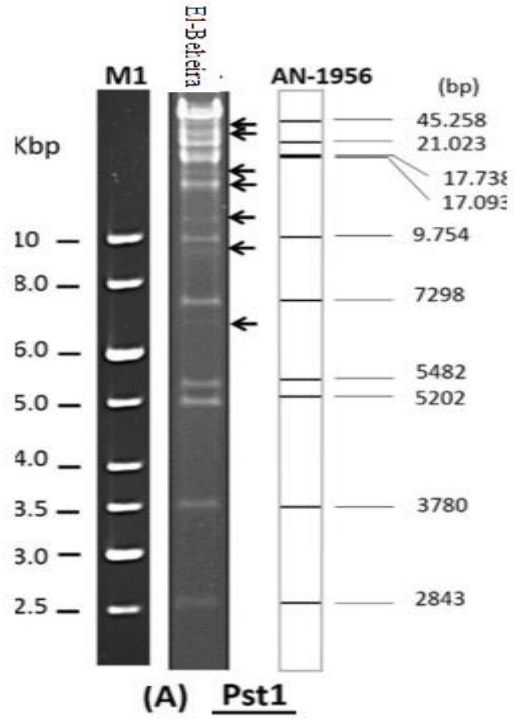

Fig. 2 Restriction fragment pattern of SpltNPV- NPV EL-Beheria and SpltNPV-AN1956 DNA using PstI.

Phylogenetic analysis

The isolate's genomic DNA was effectively extracted, and two oligonucleotide primers created to amplify a partial segment of the polyhedrin gene were employed in conjunction with the genomic DNA. These primers were used in the current work for the detection and identification of Egyptian NPVs by PCR as well as phylogenetic analysis since they are highly conserved across NPVs (Jehle et al. 2006). The outcomes demonstrated that the PCR-amplified fragments yielded the predicted size of the polygene of around 494 bp (data not shown), which was then subjected to Sanger nucleotide sequencing to be compared to other NPVs corresponding to sequences accessible in NCBI GenBank. With pairwise sequence identities of 99.04% with *S. littoralis* nucleopolyhedrovirus isolate A26-5 (Acc. No. AY706717), alignment of the NPV El-Beheira and isolates with polyhedrin gene sequences of other NPV isolates confirmed a significant degree of co-linearity within the genomes of these NPVs (Fig. 3).

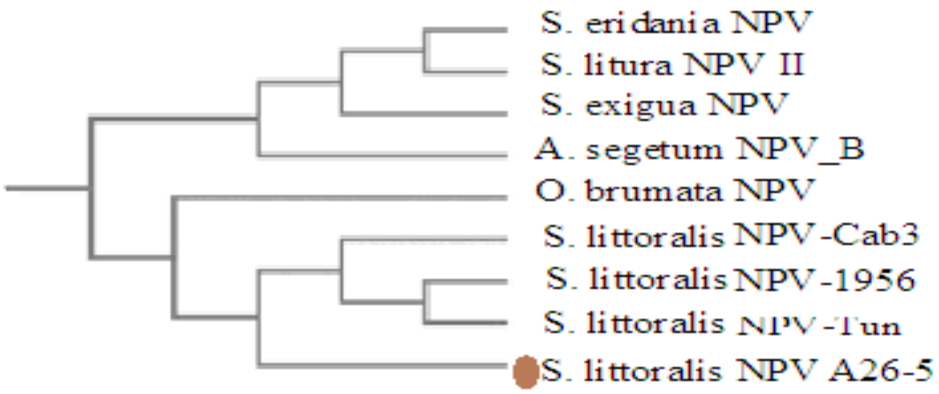

Fig. 3 Phylogenetic inference of connections among NPV isolate based on alignments of polyhedrin nucleotides. Using the neighbor-joining approach, the evolutionary history was deduced. Red dot represent the SpliNPV isolate NPV El-Beheira

Non-exposed nuclear polyhedrosis virus' insecticidal effects on 4th instar larvae

The findings showed that mortality increased with increasing nuclear polyhedrosis virus concentrations, reaching 79.96, 78.84, and 61.44%, respectively, at the initial stock concentrations for non-exposed nuclear polyhedrosis virus ($1.1x10^8$, $3.11x10^7$, and $6.7x10^6$ PIB/ml) (Table 1).

Table (1): Lethal amounts and the duration of non-exposure

Concentration (PIB/ml D.W)	No. larvae	%mortality response	LC50 (PIB/ml D.W)	LC90 (PIB/ml D.W)	Slope ±SE.	LT50 (days)	LT90 (days)
$1.1x10^8$	60	79.96	$1.3x10^6$	$6.03x10^8$	0.48±0.20	4.29	27.28
$3.11x10^7$	60	78.84				9.26	17.11
$6.7x10^6$	60	61.44				12.32	43.46

There were 1.3 x 10^6 and 6.03 x 10^8 lethal concentrations for 50 and 90%, respectively. Lethal times of 50 and 90% for concentrations of 1.1x108, 3.11x107, and 6.7x106 PIB/ml were 4.29 and 27.28 days, 9.26 and 17.11 days, and 12.32 and 43.46 days, respectively.

Effect of exposure periods to UV on the efficacy of nuclear polyhedrosis virus against 4th instar larva

According to the findings (Table 2,3,4), nuclear polyhedrosis virus exposure to UV resulted in a decrease in the number of PIB/ml, a

decrease in insecticidal activity, and a lengthened time before the death of larvae.

Table (2): Nuclear polyhedrosis virus lethal amounts and times for UV-exposed and non-exposed *A. Ipsilon* larvae

Exposed period (minutes)	Concentration (PIB/ml D.W)	No. larvae	%mortality response	LC50 (PIB/ml D.W)	LC90 (PIB/ml D.W)	Slope ±SE.	LT50 (days)	LT90 (days)
0.0	$1.1x10^8$	60	79.96	$7.7x10^6$	$5.2x10^8$	0.70 ± 0.11	4.29	27.28
5.0	$2.9x10^6$	60	35.2				18.72	59.47
20.0	$6.025x10^5$	60	24.07				21.88	125.76

Table (3): Nuclear polyhedrosis virus lethal amounts and times for UV-exposed and non-exposed *A. Ipsilon* larvae

Exposed period (minutes)	Concentration (PIB/ml D.W)	No. larvae	%mortality response	LC50 (PIB/ml D.W)	LC90 (PIB/ml D.W)	Slope ±SE.	LT50 (days)	LT90 (days)
0.0	$3.11x10^7$	60	78.84	$2.97x10^6$	$1.7x10^8$	0.73 ± 0.2	9.26	17.11
5.0	$2.9x10^6$	60	44.44				21.02	97.81
20.0	$9.4x10^5$	60	39.07				16.29	63.36

Table (4): Nuclear polyhedrosis virus lethal amounts and times for UV-exposed and non-exposed *A. Ipsilon* larvae

Exposed period (minutes)	Concentration (PIB/ml D.W)	No. larvae	%mortality response	LC50 (PIB/ml D.W)	LC90 (PIB/ml D.W)	Slope ±SE.	LT50 (days)	LT90 (days)
0.0	$6.7x10^6$	60	61.11	$6.1x10^6$	$6.2x10^7$	1.27 ±0.37	12.32	43.46
5.0	$4.1x10^6$	60	28.32				18.74	54.66
20.0	$1.5x10^6$	60	25.93				25.05	105.52

Results revealed that a concentration of $1.1x10^8$ PIB/ml decreased to $2.9x10^6$ and $6.125x10^5$, respectively, when exposed to UV for 5.0 and 20.0 minutes, as well as the other concentrations.

Discussion:

These findings demonstrated a positive relationship between concentration and statistically different $LC_{50,90}$ & $LT_{50,90}$ values. Adel El-Said hatem et al. (2011) confirmed that the median lethal times were 28.0, 27.0, and 24.7 days for the concentrations of 1.12 x 10^8, 5.6 x 10^8, and 2.8 x 10^9 OB/ml, respectively, and mortality increased with Sp.li.GV concentrations. The LT_{50} at similar response levels were 27.9, 21.9, and 15.9 days, respectively, for L1, L3, and L5 instars. Shaurub et al. (2014) found that with LC_{25} and LC_{50} values of 10.10× 10^6 and 8.43× 10^8 PIB/ml distilled water, respectively, and a rise in viral concentrations, the mortality of *A. ipsilon* fourth larval instars increased developmentally. Sp.li.GV's effectiveness in *A. ipsilon* larvae was lower than that of other GV-host systems. The LD50s for larvae of *Lacanobia oleracea* treated with LoGV varied from 101.4 OB/larva in L1 instar to 107.45 OB/larva in L5 instar, according to Matthews et al., (2002). The majority of Lepidoptera baculoviruses that have been studied have shown significant instar-to-instar variations in vulnerability (Hochberg, 1991; Sait et al. 1994; Teakle et al. 1986; Sporleder et al, 2007). As with *Spodoptera exigua* infected with Sp.ei. NPV isolated from California, the Sp.li.GV LT50s decreased with rising dose values in each instar. (Smith, 1987). NPV disrupted juvenile hormone rates in the hemolymph, the presence of the enzyme ecdysteroid UDP-glucosyltransferase (EGT), which is required for the regulation of host insect metamorphosis because EGT inactivates ecdysteroids by conjugating them with galactose, and many other factors, according to Subrahmanyam and Ramakrishnan's (1980) research, which suggested that the failure of infected larvae to molt to pupal stage. Also, the virus egt gene expression, as seen in the inability of lethally infected *L. oleracea* larvae to undergo moulting (Smith and Goodale 1998). However, Nakai et al., (2004) observed that Sp.li.GV infection prevented *S. littoralis* from metamorphosing from a larva to a pupa, but not from a larva to larval moult.

Insect pathogens called baculoviruses are typically discovered when outbreak levels in their hosts are reached and a disease epizootic takes place. They are bio-insecticides that are safe and effective against a variety of nuisance insects, particularly lepidopterous insects. Their sensitivity to restrictions like ultraviolet light limits their use in the control of insect pests. Baculoviruses are powerful biological control

agents against a number of significant lepidopterous parasites (Arivudainambi et al, 2000: Baskaran, 2007). However, the UV spectrum of sunshine quickly inactivates baculoviruses, with the majority having a half-life of two to several hours (Elnagar & Abul-Nasr, 1980; Villamizar et al., 2009). Therefore, one of the most crucial criteria for evaluating a protective substance is field stability. UV from the sun could make a virus ineffective when used as a biocontrol agent in the outdoors. As a result, the virus application in the environment has a limited duration of action against the intended pests. According to El-Helaly et al. (2013), when SpliNPV was used as a the only treatment, the recorded rates of mortality among *S. littoralis* neonate larvae were 96.00%, 48.78%, 6.97%, 0%, and 0%, respectively, after exposure periods of 10, 24, 48, 96, and 168 hours to natural conditions (under UV), as opposed to 100.00% in the case of a unirradiated virus (The LT50 determined was only 24.07 hours). Baculoviruses were quickly rendered inactive in the field after exposure to SUV or natural sunlight, according to earlier research (El Salamouny et al., 2000; Khattab, 2003). Purified virus suspension was found to be less efficient than crude extract under UV light due to the latter's presence of colouring agents (Elnagar and Abul-Nasr, 1980). The most effective microbial insect pest control agent is nuclear polyhedrosis virus, but its action is still constrained due to the virus's degradation in field conditions. (UV radiation). To enhance the persistence of the virus in the opposite harsh sunny conditions in Egypt, it is necessary to have some virus protection, such as natural UV protectant (based on some plant derived materials).

Conclusion

In light of all of that, the Nuclear Polyhedrosis Virus is crucial for its insect host and is safe for the ecosystem and all living things, including people. Therefore, it is important to research the issues that are affecting it in order to understand how to use this virus as an option to toxic chemical insecticides. This article examined the effects of the UV-exposed Nuclear Polyhedrosis Virus (NPV) on the black cutworm Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae) and confirmed the need to find a safer alternative to chemical insecticides that cause cancer. It also suggested using the Nuclear Polyhedrosis Virus as that safer

alternative because of its specificity to its insect host and because it is not carcinogenic.

Abbreviations

PIB/ml: Polyhedral Inclusion Bodies per milliliter; UV: Ultraviolet; LT: Lethal times; LC: Lethal concentration; OBs:Occlusion Bodies

Declarations

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Conflicts of interest/Competing interests

I declare that they have no conflict of interest.

Availability of data and material

The data supporting the results reported in a published article can be found - including, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study.

Code availability

I confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

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