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Effect of gamma irradiation on the susceptibility of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) to the infection with nucleopolyhedrosis virus

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Abstract

The sensitivity of irradiated cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), to infection with nucleopolyhedrosis virus (SpliNPV) was evaluated. *S. littoralis* pupae were irradiated by four low doses of gamma radiation, 40, 60, 80, and 100 Gy, and the sensitivity to viral infection of the resultant F_1 larvae was evaluated. The results indicated that the irradiated F_1 larvae showed high sensitivity to different SpliNPV concentrations. In the case of 1×10^3 PIBs/ml concentration, the mortality percentages of F_1 larvae drastically increased to 25.14, 46.53, 93.2, and 91.3% at the doses 40, 60, 80, and 100 Gy, respectively, in comparison to 4.9% for the un-irradiated treatment. The results revealed that the numbers of deposited eggs, hatched eggs, and survived larvae and pupae were reduced at all the radiation doses as compared to the control treatment. The results indicated that 40 and 60 Gy were the effective doses for irradiating *S. littoralis* male pupae to produce F_1 larvae very sensitive to SpliNPV which may help in baculovirus mass production.

Keywords: *Spodoptera littoralis*, Gamma irradiation, Baculovirus, Sensitivity, Synergistic effect

Background

Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) is an economically important polyphagous pest in Egypt. It was reported to attack a wide range of food plants (112 cultivated plants belonging to 44 families world wide and 60 plants in Egypt) causing serious economic losses in many crops (Abd El-Razik and Mostafa 2013). A multifaceted approach is required because of the many records of resistance developing in this insect to several groups of pesticides (Ramakrishnan et al. 1984 ; Armes et al. 1997). Microbial insecticides such as baculoviruses are environmentally safe and selective bio-insecticides and can be used as alternatives to chemical pesticides (Armenta et al. 2003). Baculovirus products are commercially available with trade names for use in certain parts of the world (Black et al. 1997); however, the use of one or a combination of techniques to increase the efficacy of baculovirus production is still needed. The efficacy of NPV may be improved if any factor goes in line with its mode of action, such as effect on the peritrophic membrane, epithelial cells of the midgut, or depression of the insect immune system. However, many studies have been attempted to increase the efficiency of virus production, including the use of artificial diets (Chen et al. 2000; Gupta et al. 2007; Elvira et al. 2010), specific inoculum dose and stage of inoculation (Narayanan and Jayaraj 2002), and the optimization of rearing temperature and harvesting time of infected insects (Cherry et al. 1997; Subramanian et al. 2006). The reduction in production time or the use of alternative hosts or vectors may also enhance the efficiency of baculovirus production (Monobrullah et al. 2007; Beek and Davis 2007).

It was noticed that gamma radiations can cause deleterious effect on reproductive potential and is mainly

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used in insect control programs. There are two effects of gamma irradiation on the cells; the first is the direct effects such as clustered DNA damage and DNA double-strand breaks, and the second is the indirect effect causing DNA damage by the induction of reactive oxygen and free radicals. In the case of low doses, the direct effect is lower than the indirect one; therefore, the effects are stochastic and depend mainly on the efficiency of the stress response's protective mechanisms and are known to induce hormesis (Moskalev et al. 2011). Low doses of gamma radiation affect the processes of cell proliferation and differentiation, causing DNA damage, apoptosis, proteolytic degradation, autophagy, and oxidative stress. Also, it has an impact on the immune response and the development of the organism; changes the metabolism of proteins, lipids, fatty acids, amino acids, and hormones; and alters energy metabolism leading to changes in the cell cycle (Feinendegen 2005; Seong et al. 2011; Zhikrevetskaya et al. 2015).

Irradiated *Ceratits capitata* and *Anastrepha ludens* exhibited signs of damage to midgut tissue, cellular organelles, and peritrophic membrane formation. In addition, bacterial growth appeared diminished in the midguts of irradiated flies compared to un-irradiated ones (Lauzon and Potter 2012).

Therefore, this study aimed to evaluate the use of gamma irradiation against the cotton leaf worm, *S. littoralis*, as a synergistic factor for increasing NPV yield.

Materials and methods

Bioassay analysis

A local isolate of *S. littoralis* multiple embedded nucleopolyhedrosis virus (SpliNPV) was used in the experimental studies originally isolated in Egypt by Abul Nasr (1956). Concentration-mortality regressions were calculated, using three different virus concentrations. The SpliNPV 6×10^9 PIBs (polyhedral inclusion bodies)/ml inoculum was diluted in distilled water and the suspension was adjusted to contain 1×10^2, 1×10^3, 1×10^4, 1×10^5, and 1×10^6 BIPs/ml. The effect of gamma irradiation, followed by SpliNPV application, was evaluated in comparison to SpliNPV alone on the neonate and third-instar larvae of *S. littoralis*. Bioassay tests were repeated in five replicates with 50 larvae per treatment.

Irradiation technique

Full-grown male pupae of *S. littoralis* were irradiated by 0, 40, 60, 80, and 100 Gy, using Cobalt-60 gamma cell. This source is located at the cyclotron project, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt. The dose rate of the source was 7.0 Gy/min.

Biological studies

S. littoralis was obtained from a laboratory culture maintained at 27 ± 2 °C and 65% RH. The colony was maintained on a semi synthetic diet (Shorey and Hale 1965).The emerged male moths from all irradiated treatments were allowed to mate with un-irradiated female moths to obtain the F_1 generations. The number of eggs laid per female, percent of egg hatching, and mortality of larvae and pupae were recorded. Five replicates per treatment were performed.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and the means were separated using Duncan's multiple range test ($P = 0.05$) (Steel and Torrie 1960).

Results and discussion

The results revealed that the treatment of neonate larvae with 1×10^2 PIBs did not lead to virus production where this low concentration resulted in few dead larvae at the different doses applied. Also, the highest virus concentrations of 1×10^5 and 1×10^6 PIBs caused (90 to 100%) mortality in this early larval stage, but led to no virus yield (Table 1). While, the treatment of neonate larvae with 1×10^3PIBs caused the highest yield of virus as the treated larvae died in the fifth instar.

The mortality of third-instar larvae treated with the SpliNPV concentration of 1×10^2 PIBs/ml increased significantly to 8.7, 10.73, 10.46, and 10.53% at the doses of 40, 60, 80, and 100 Gy, respectively, as compared to 0.4% for the un-irradiated larvae (Fig. 1). For the 1×10^3 PIBs/ml concentration, the percentages of mortality increased significantly to 25.14, 46.53, 93.2, and 91.3% at the same doses, respectively, as compared to 3.9% for the un-irradiated treatment (Fig. 1). In the case of 1×10^4 PIBs/ml concentration, the mortality percentages of F_1 larvae drastically increased to 98.7, 99.0, 100.0, and 100.0% at the doses 40, 60, 80, and 100 Gy, respectively, as compared to (7.0%) for the un-irradiated treatment (Fig. 1).

These results indicated that there was no clear difference in SpliNPV pathogenicity to F_1 neonate larvae from irradiated pupae with different doses. These findings

Table 1 Concentration-mortality response of neonate *Spodoptera littoralis* larvae resulted from mated females with irradiated males as pupae followed by SpliNPV treatments

SpliNPV PIB/ml	1×10^3	5×10^3	1×10^4	5×10^4	1×10^5	5×10^5	1×10^6
Dose (Gy)							
0	18.12a	29.72a	41.89a	85.23a	87.23a	93.33a	99.33a
40	16.66a	26.71a	45.63a	90.00a	89.86a	96.59a	100.00a
60	17.02a	31.03a	44.89a	94.55a	91.15a	97.94a	100.00a
80	17.44a	26.17a	45.27a	88.51a	87.11a	95.91a	99.31a
100	17.00a	26.35a	46.93a	85.03a	88.03a	95.94a	99.32a

Means designated with the same letter in the same column are not significantly different ($P \geq 0.05$)

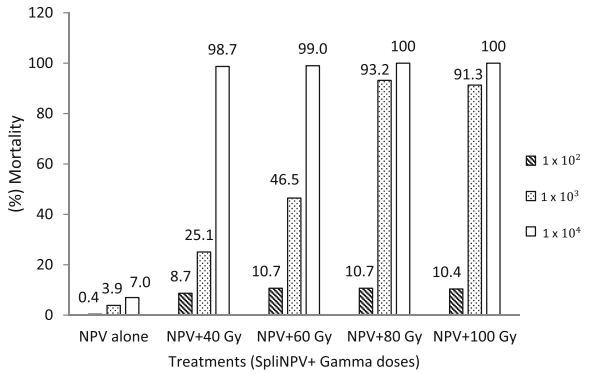

Fig. 1 Effect of 1×10^2, 1×10^3, and 1×10^4 PIBs/ml concentrations of SpliNPV on *S. littoralis* third-instar larvae resulted from males irradiated as full-grown pupae with sub-sterilizing doses 20, 40, 60, and 80 Gy in comparison to un-irradiate ones (virus alone). Data are expressed as mean values of five replicates per treatment

may be due to high sensitivity of neonate larvae to the NPV (Narayanan and Jayaraj 2002; Beek and Davis 2007). This high sensitivity made the morality of neonate larvae by NPV high in both the control (treated with virus only) and the irradiation and virus treatments. In contrast, the third-instar larvae were found to have low sensitivity to NPV, especially at the lower concentrations (1×10^2, 1×10^3, and 1×10^4 PIBs/ml).

The NPV mass production was influenced by several factors such as virus concentration, larval age at virus treatment, temperature, and harvesting time (Cherry et al. 1997; Subramanian et al. 2006; Rios-Velasco et al. 2012). Furthermore, numerous techniques have been proposed to improve the production of baculoviruses such as juvenile hormones used for increasing the production of *Spodoptera exigua* multicapsid NPV (SeMNPV) (Lasa et al. 2007) and for the production of *Spodoptera litura* NPV (SpltNPV) (Liao et al. 2016). In addition, Elvira et al. (2010) developed a low-cost diet for the large scale in vivo production of SeMNPV.

The present study investigated also some biological parameters of *S. littoralis* that could be affected by the low doses of gamma radiation applied to increase NPV production. Data in Table 2 shows that the number of eggs was insignificantly decreased as the gamma dose increased, while the percentages of egg hatching were significantly reduced at the tested doses than in the control treatment and the greatest reduction was recorded at the dose of 100 Gy. In the case of 80 and 100 Gy, the larval duration elongated to 14.6 and 15.1 days, respectively in comparison with (14.1 days) for the un-irradiated treatment. The percentages of larval and pupal mortality increased significantly at all gamma doses. These results go in line with those obtained by Carpenter et al. (1986), Seth and Sehgal (1993), Yousef (2001), and Abass et al. (2017).

Table 2 Effect of gamma irradiation on the reproductive biology of *Spodoptera littoralis* irradiated as full-grown male pupae

Doses (Gy)	No. of eggs (Av)	Egg hatch (%)	Larvae duration/ days (Av)	Pupal duration/ days (Av)	Larval mortality (%)	Pupal mortality (%)
0	1414.4a	89.0a	14.1a	10.0a	5.2a	3.4a
40	1407.5a	80.1b	13.8a	10.3a	13.5b	10.2b
60	1397.3a	67.2c	13.9a	10.2a	20.5c	16.1c
80	1354.4a	65.7c	14.6b	10.5a	22.3c	15.2c
100	1361.2a	45.3d	15.1c	10.6a	36.1d	17.2c

Means designated with the same letter in the same column are not significantly different ($P \geq 0.05$)

Conclusions

The present study suggested that the two doses of 40 and 60 Gy were the effective doses for irradiating *S. littoralis* male pupae to produce very sensitive F_1 larvae to SpliNPV. Furthermore, the two doses of gamma radiation slightly affected the reproductive biology of *S. littoralis*, while the first generation larvae resulted from irradiated full-grown pupae were more susceptible to SpliNPV than un-irradiated ones. These finding may help in the mass production of baculoviruses.

Abbreviations

BIPs: Polyhedral inclusion bodies; SpliNPV: *Spodoptera littoralis* multiple embedded nucleopolyhedrosis virus

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Availability of data and materials

The authors declare that they have no objection to the availability of data and materials.

Authors' contributions

The authors contribute equally to this work, both authors carried out the bioassay and biological studies. AMAE conducted the isolation, propagation of the baculovirus, and prepared the virus suspension in order to bioassay it. WAAS carried out the irradiation treatment and statistical analysis. Both authors contribute in the experimental design and writing the manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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