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Impact of simultaneous treatment of gamma irradiation and *Bacillus thuringiensis* on cotton leaf worm *Spodoptera littoralis* (Boisd.) (Noctuidae: Lepidoptera)

Waheed A. A. Sayed*, Reda Sayed Hassan and Thanaa Mohamed Sileem

Abstract

Background: The two biological control methods, inherited sterility technique (IST) and *Bacillus thuringiensis* (*Bt*), are considered as the two promising methods for lepidopteran pest management. Simultaneous treatment with both techniques was assessed against the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Noctuidae: Lepidoptera) compared to individual treatment for developing the lepidopteran management program.

Results: Pupation and adult emergence were significantly lower in simultaneous treatment than irradiated insects individually. Larval mortality test estimated a LC_{50} of 1.6 × 10^4 IU/mg (*Bt*), in which decreased to 1.5 × 10^4 IU/mg (*Bt and* 75 Gy), and 7.7 × 10^3 IU/mg (*Bt and* 125 Gy). Insect survival was prolonged for both individual and simultaneous treatments. The ratio of males was 3.5-fold to females in 1.5 × 10^4 IU/mg and 75 Gy, while threefold of males to females was recorded in the treatments of 3.75 × 10^3 IU/mg and 75 Gy, 7.5 × 10^3 IU/mg and 75 Gy, 7.5 × 10^3 IU/mg and 125 Gy and 1.5 × 10^4 IU/mg and 125 Gy. Results of fecundity, fertility and sperm transfer of F_1 males were significantly lowered in irradiated insect combined with *Bt* than individual treatment.

Conclusion: Gamma irradiation treatment combined with *Bt* concentrations achieved a high reduction of *S. littoralis*, and the toxicity effect of *Bt* increased as compared with individual treatments taking advantage as compatible control tactics. Simultaneous treatment of IST and *Bt* could be considered as a competent approach for *S. littoralis* population suppression.

Keywords: Inherited sterility technique, *Bacillus thuringiensis*, *Spodoptera littoralis*, Gamma irradiation, Biocontrol

Background

Over the last decades, attempts to promote safe and sustainable alternative control methods have been intensively studied and interestingly engrossed, whereas the extensive use of conventional pesticides against lepidopteran insect pests has had adverse impacts on the environment and its fauna (Mulé et al. 2017). The program that incorporates various biocontrol techniques synchronously for growing healthy crops and avoiding pesticide hazardous has been approved globally, which is the concept of promising integrated pest management (Singh et al. 2019). Evidence, insect resistance against several control agents, is a critical point for the design of an effective control programmer. Simultaneous treatment protocol brings regenerative pest management a step closer for a successful program against targeted insect pests in particular that developed resistance to control agents. Sterile insect technique (SIT) is a promising genetic method for pest population suppression, based on releasing sterile insects systemically in area wide after

*Correspondence: waheed.sayed@eaea.org.eg

Biological Applications Department, Nuclear Research Center, Egyptian Atomic Energy Authority, Cairo ET-11787, Egypt

being exposed to Ionizing radiation (Dyck et al. 2021). Lepidoptera are radioresistance requiring high doses to induce sterility in adult moths. These sterilizing doses cause deleterious effect of irradiated insect that reduce the efficacy of sterile moths to compete the wild type in nature (Marec and Vreysen 2019). Consequently, inherited sterility technique (IST) offers significant advantages over classical sterile release method for lepidopteran pests, and sterility in the F_1 generation is induced by irradiating the parents with sub-sterilizing doses which could be partial sterility (Bloem et al. 2003). Although IST has been reported as an effective technique for lepidopteran population suppression, it was supposed that it is a complementary component of an area-wide and sustainable long-term strategy in the pest management programs rather than a stand-alone technique (Suckling et al. 2017). Simultaneous treatment of IST with another compatible control method could improve the SIT program efficacy (Benedict 2021) that it is essential to suppress the population density of insects to a level, which makes it economically feasible to release a dominant population of the sterile insect over an extended period. Various biorational pesticides have been suggested to use in combination with SIT for pest management (Howse et al. 2007). Biocontrol methods can be stimulated as a supplementary control tool to IST with regard to an environmentally safe, effective and alternative method. Attempts have been conducted to use agents in combination with irradiation treatment such as the predator on *Tuta absoluta* (Cagnotti et al. 2016), the parasitoid on *Spodoptera exigua* (Hübner) (Carpenter and Sheehan 1996), the Baculovirus on *Spodoptera littoralis* (Sayed and El-Helaly 2018), the entomopathogenic nematode on *Galleria mellonella* (Salem et al. 2020) and the entomopathogens fungi on *S. littoralis* (Gabarty et al. 2019). Recently, *Bacillus thuringiensis* (*Bt*) is considered one of the most effective entomopathogenic against lepidopteran pests (Raymond et al. 2010). It is widely used in many regions over the world due to its harmless to vertebrate, non-target organism and other ecosystems (Bravo et al. 2011). Both IST and *Bt* offer a way to equate the two separate treatments with a simultaneous one. Both treatments could be used against lepidopteran insects effectively but are adjusted with regard to the different conditions of operation (e.g., time, doses and species). It has been found that the use of IST program resulted to suppressing resistance of pink bollworm, *Pectinophora gossypiella (*Saund**.)** to genetically engineered cotton crop contains *Bt* (Tabashnik et al. 2010). The advantages of this combination treatment have been demonstrated by the economic benefits achieved (Tabashnik et al. 2021). The cotton leaf *S. littoralis* is one of the most destructive lepidopteran pests in tropical and subtropical area that cause damage to more than 100 different crops and vegetable plants considering it as an economic pest in different countries (Sayed et al. 2021). In this context, the present trial aimed to provide assessment of the simultaneous treatment, *Bt* and IST as promising methods for *S. littoralis* management, which has a multiple regression optimization perspective, taking into account the effective dose, insect response and interaction of the two techniques.

Methods

Insect

The colony of cotton leaf worm, *S. littoralis*, was maintained under laboratory conditions at 25 ± 2 ^{0}C and 65% R.H. Full-formed pupae were placed in an adult rearing cage ($40 \times 20 \times 10$ cm) with small cups, containing pieces of cotton wool soaked with 10% sugar for feeding the emergent moths. Egg masses were collected daily, and treated with a formalin (10%) for surface sterilizing against pathogens as suggested by (Connell 1981), and then, they were transferred to glass gars (1600 cc) covered with muslin cloth until hatching. The newly hatched larvae were transferred to rounded plastic bowls (70×15 cm) provided with semi-artificial diets that were developed by (Sayed et al. 2021) and covered with both muslin cloths until pupating.

Irradiation

The source of gamma radiation used during the present study was from a Cobalt 60 (^{60}Co) irradiator; the dose rate of irradiation source was 407.2 Gy/h. The full-grown male pupae of *S. littoralis* were irradiated (24–48 h before emergence of adults) with sub-sterilizing doses of gamma radiation (75 and 125 Gy) (Sayed and El-Helaly 2018). After the adult emergence, irradiated adult males were paired with untreated adult females. A group of untreated pairs were used as control. The deposited eggs were collected to continue the F_1 generation.

Bacillus thuringiensis

The commercial product of *Bacillus thuringiensis*, *aizawai* (*Bta*) strain (XenTari, ABTS-1857, 540 g/kg) at 15,000 international unit IU per mg was used in this study. Stock solution of the compound was prepared in tap water and then diluted by water to series of concentration solutions 3.75×10^3, 7.5×10^3, 1.5×10^4, 3.0×10^4 and 6.0×10^4 IU/mg. Newly molted 3rd larvae were treated with *Bta* by feeding on a semi-artificial diet treated with the previous concentrations. One milliliter of each concentration was spread on the surface plate ($20 \times 10 \times 3$ cm) containing 50 ml of different semi-artificial diets, and each treatment was repeated in five replicates with 50 larvae each. The mortality responses of larvae were daily recorded.

Bioassay studies

The F_1 larvae from P_1 male irradiated as full-grown pupae with 75 and 125 Gy were divided into two groups; the first was treated as 3rd larvae with different concentrations of *Bta*, while the second group was left without *Bta* treatment for comparison. Moreover, another group of un-irradiated larvae was treated by *Bta* only. In addition, a group of untreated control was left for comparison. Larval mortality and larval duration, pupal duration, pupation, emergence, surviving from larvae to adult emergence and sex ratio were calculated for all individual and combined treatments. Resulted male moths from each treatment were paired with un-irradiated female moths, whereas the number of eggs/female and egg hatching were recorded. Moreover, numbers of mating/female were assessed upon death, and the female moths were dissected to estimate the number of sperm transferred (Spermatophores in the bursa copulatrix). Five replicates were used for each treatment. Each replicate consisted of 20 larvae.

Statistical analysis

Mortality response of various treatments either *Bt* concentrations or simultaneous treatment of *Bta* and gamma radiation doses (75 and 125 Gy) was tested using the Probit analysis which were expressed as a percentages, and the median lethal concentration (LC_{50}) was estimated. Analysis of variance (ANOVA) technique was used to analyzed the data of biological studies and the averages were analyzed using Tukey's range test (F-tests) ($P=0.05$) (Gurvich and Naumova 2021). Data of egg hatch, pupation and emergence (%) were transformed by arcsine tables, while the means and standard errors were from original data.

Results

Mortality response assay of *S. littoralis* larvae

Figure 1 presents the mortality responses of treated *S. littoralis* larvae to *Bta* concentrations. The LC_{50} was 1.6×10^4 IU/mg, and this response was slightly increased for F_1 larvae irradiated with 75 Gy, where the LC_{50} was 1.5×10^4 IU/mg, while the toxicity was highly increased for F_1 larvae irradiated with 125 Gy (twofold), since the LC_{50} was 7.7×10^3 IU/mg. Based on these results, there was a positive interaction effect between the *Bta* concentrations and sub-sterilizing doses of gamma irradiation, where the F_1 larvae were more susceptible to the *Bta* than to the un-irradiated ones.

Bioassay studies

Regarding the mortality response studies, the *Bta* concentrations (3.75×10^3, 7.5×10^3 and 1.5×10^4 IU/mg) were selected to further experiments because no yields (pupae and adults) were obtained at 3.0×10^4 and 6.0×10^4 IU/mg. Survivals of remaining alive larvae of various treatments, irradiation, *Bta* and simultaneous treatments are listed in Fig. 2. In female line, the durations of larvae were significantly prolonged at 7.5×10^3 IU/mg, 1.5×10^4 IU/mg, 125 Gy and all simultaneous treatments compared to 75 Gy, 3.75×10^3 IU/mg and control treatment ($F_{11,59}=58.1$, $p<0.0001$). The same trend was recorded in male line, whereas the duration reached 21.06 days of

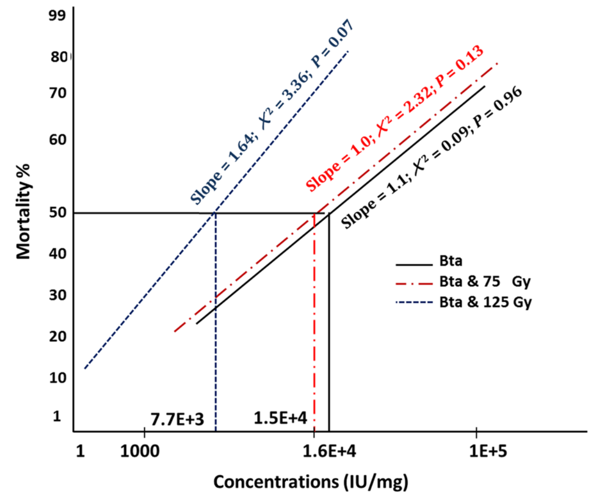

Fig. 1 Mortality response of *Spodoptera littoralis* larvae treated with *Bta* concentrations (IU/mg), simultaneous treatments of *Bta* concentrations and 75 Gy and simultaneous treatments of *Bta* concentrations and 125 Gy

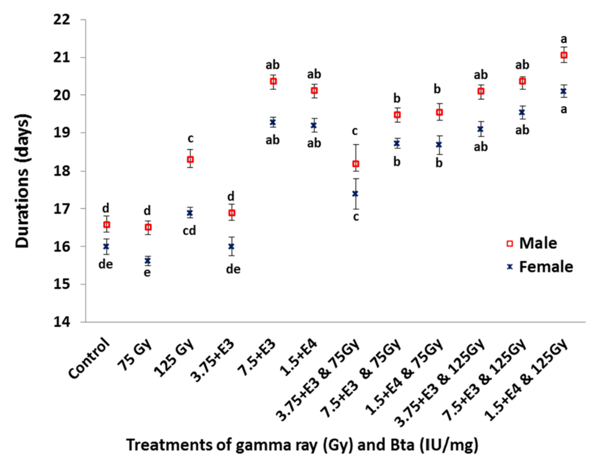

Fig. 2 Larval durations of *Spodoptera littoralis* F_1 progeny resulting from irradiated pupae with gamma radiation doses (Gy), treated with *Bta* concentrations (IU/mg) and simultaneous treatments of *Bta* concentrations and gamma radiation doses

1.5×10^4 IU/mg and 125 Gy as compared to 16.25 days of control treatment ($F_{11,59} = 49.2$, $p < 0.0001$).

Results of pupation and adult emergence resulted from different treatments showed that the percentages of pupation and adult emergence were reduced gradually by increasing the *Bta* concentration levels and higher significant reductions were caused in simultaneous treatment than the individual and control treatments. Data of the percentage of larvae to reach pupae were highly significant between the different treatments ($F_{11,59} = 298.5$, $P < 0.0001$), where low pupation percentages (35.06, 56.26, 57.6 and 61.9%) were recorded in the treatments of 1.5×10^4 IU/mg and 125 Gy, 1.5×10^4 IU/mg, 3.75×10^3 IU/mg and 125 Gy and 1.5×10^4 IU/mg and 75 Gy, respectively, as compared to the high pupation percentages (95.2, 80.3 and 80.2%) of control, 75 Gy and 125 Gy (Fig. 3). In the same table, adult emergence percentages were highly significant among the different treatments ($F_{11,59} = 561.4$, $P < 0.0001$), where the adults were emerged in low percentage (14,9%) of 1.5×10^4 IU/mg and 125 Gy as compared to (80.1, 80.5, 70.3 and

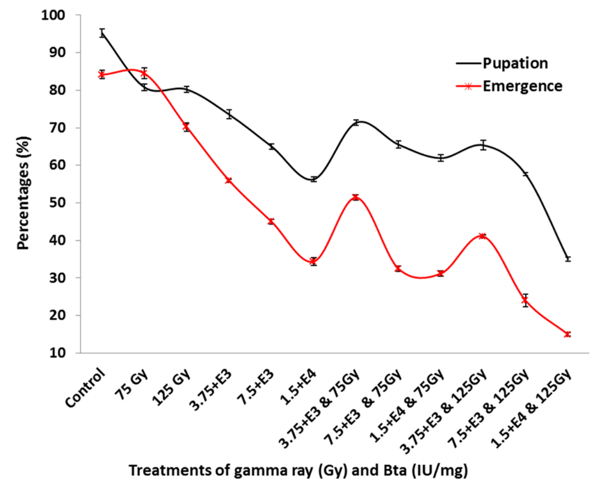

Fig. 3 Pupation and adult emergence of *Spodoptera littoralis* F_1 progeny resulting from irradiated P_1 pupae with gamma radiation doses (Gy), treated with *Bta* concentrations (IU/mg) as larvae and simultaneous treatment of *Bta* concentrations and gamma radiation doses

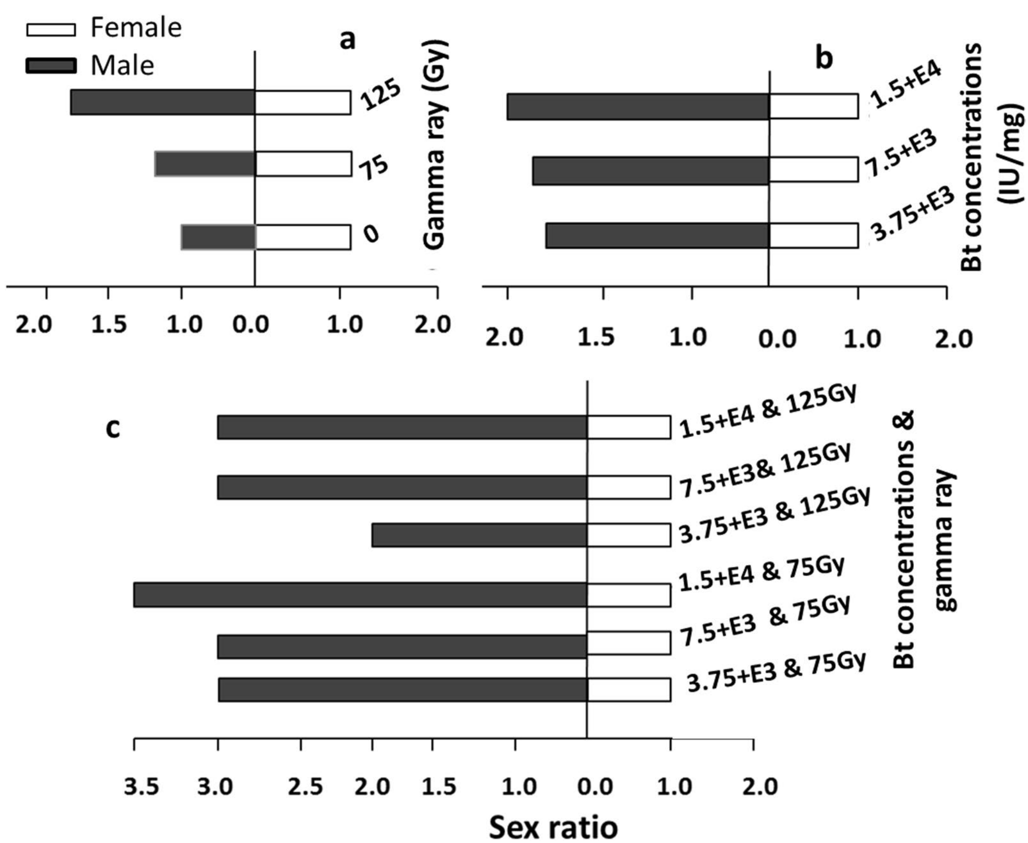

Fig. 4 Sex ratio of *Spodoptera littoralis* F_1 progeny resulting from irradiated P_1 pupae (**a**), treated with *Bta* concentrations as larvae (**b**) and simultaneous treatments of *Bta* concentrations and gamma radiation doses (**c**)

34.4%) of control, 75 Gy, 125 Gy and 1.5×10^4 IU/mg, respectively.

The sex ratio of males to females of F_1 adults varied in different treatments, and the males/females ratios were highly increased to males by increasing the gamma irradiation doses (Fig. 4a), respectively, and *Bta* concentrations (Fig. 4b), respectively, as compared to 1:1 in the control (0) treatment. Figure 4c shows that the highest ratio (3.5:1) of males/females was found in the 1.5 × 104 IU/mg and 75 Gy as compared to (3:1) of 3.75×10^3 IU/mg and 75 Gy, 1.5×10^4 IU/mg and 75 Gy, 7.5×10^3 IU/mg and 125 Gy and 1.5×10^4 IU/mg and 125 Gy.

The averages of eggs per female were reduced gradually among the different treatments ($F_{11,59} = 10{,}511.6$, $p < 0.0001$) (Fig. 5a), Similarly, the egg hatching significantly decreased than the control treatment ($F_{11,59} = 1304.3$, $p < 0.0001$) (Fig. 5b). Non-significant differences were recorded in the mating between different treatments ($F_{11,59} = 10{,}511.6$, $p = 0.0411$) (Fig. 5 c).

Discussion

The study aimed to develop the approach of simultaneous IST and *Bta*, which could potentially be applied for *S. littoralis* management. Given the above data and considering the desire to develop environmentally promising approach, simulations treatment of IST and *Bta* could be used as a novel protocol for control lepidopteran pests effectively. The noteworthy difference in the toxicity of *Bta* combined with gamma radiation doses comparing with individual treatment in the present study goes in line with (AMER et al. 2012) who found that the gamma radiation doses 150, 250, 350 reduced the LC_{50} of *Bt*. Moreover, (Sayed and El-Helaly 2018) found that the low doses 40 and 60 Gy were highly effective on the

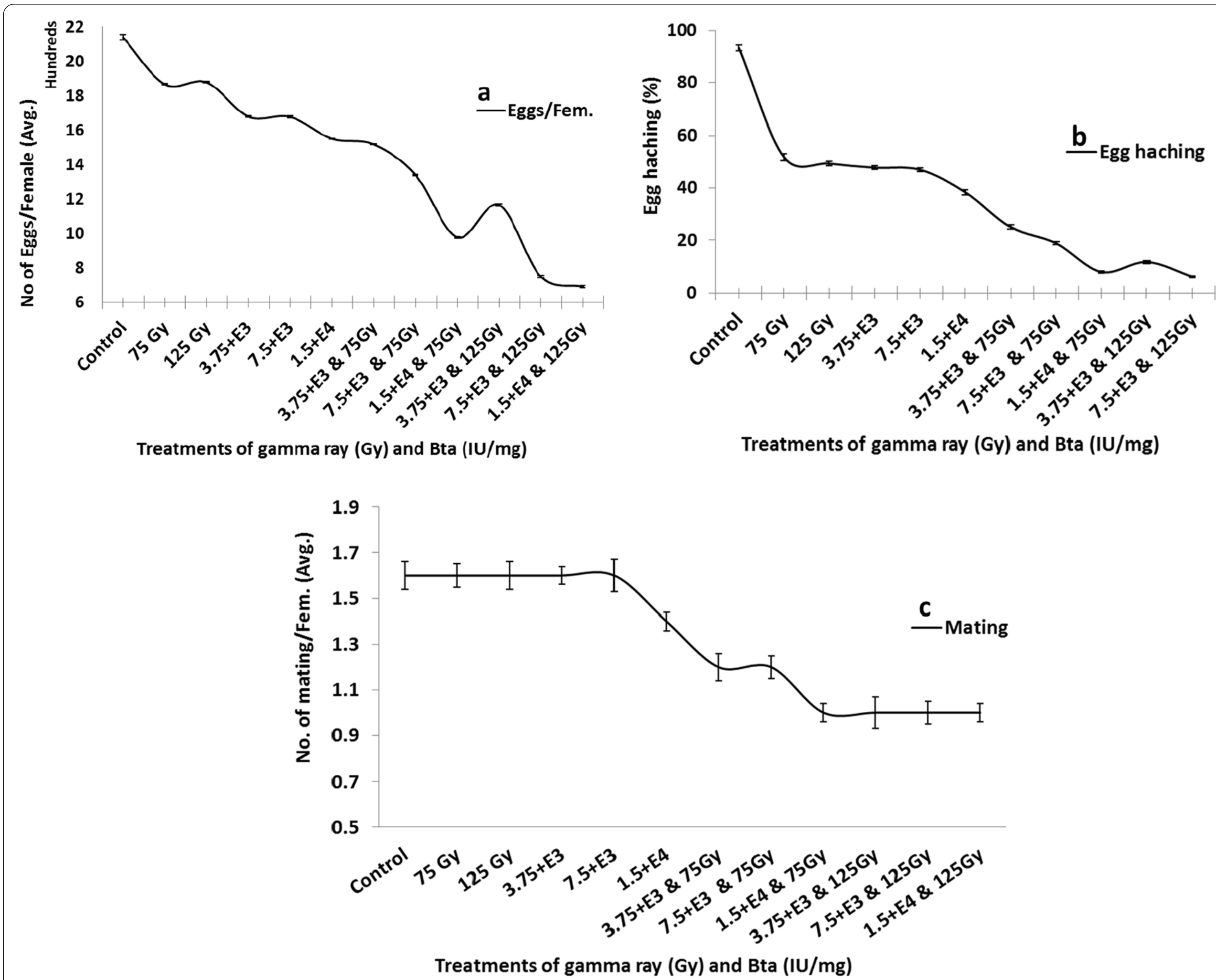

Fig. 5 Number of egg/female (**a**), (%) egg hatch (**b**) and (Avg.) number of mating/female (**c**) of *Spodoptera littoralis* F_1 adults resulting from irradiated P_1 pupae, treated with *Bta* concentrations as larvae and simultaneous treatment of *Bta* concentrations and gamma radiation doses

S. littoralis F_1 larvae to be more susceptible to *Spli*MNPV than un-irradiated ones. Synergistic effect has been noticed in the combination of *Bt* with Chlorantraniliprole as well as reduced the resistance of diamondback moth (Shabbir et al. 2021).

Results of the duration of larval stage to reach pupae revealed that the larval duration was extended in various treatments, and these data are coincident with (Carpenter et al. 2001) on *Cactoblastis cactorum* and (Walton and Conlong 2016) on *Eldana saccharina* who found an extended time of larvae to reach pupae as a result of irradiated male with sub-sterilizing doses of gamma radiation. Moreover, it has been observed prolonged of *Helicoverpa armigera* larvae with the low concentrations of *Bt* (Mohan et al. 2008).

Obtained findings revealed that the insect fitness was reduced at the low concentration of *Bta* which agree with Eizaguirre et al. (2005) on *Sesamia nonagrioides*, *Spodoptera frugiperda* (Sousa et al. 2016) and *Plutella xylostella* (Zhu et al. 2016).

Sex ratio was more declined at the favor of male at most simultaneous treatments than either control or individual treatments. These results are in line with (Bloem et al. 2003) who shifted in favor of males in the F_1 sex ratio of false codling moth, *Cryptophlebia leucotreta* irradiated with sub-sterilizing doses of gamma radiation. Obtained results revealed that there was a significant reduction in the F_1 population of *S. littoralis* derived from the combination treatment than individual one. Moreover, the results for the impact of sublethal concentrations of *Bt* are in line with that finding by (Fathipour et al. 2019) on *Helicoverpa armigera* and (Camacho-Millán et al. 2017) on *Diatraea considerate.* The finding indicated that sublethal concentrations of *Bt* caused significant fitness costs in *S. littoralis* which coincident with (Sedaratian et al. 2013) who observed a low rate of *H. armigera* development exposed to low concentrations of *Bt*. Synergistic effect was reported by (Magholifard et al. 2020) on *S. littoralis* using *Bt* and *Spli*NPV.

Conclusion

Simulations treatment of IST and *Bta* was assessed against *S. littoralis* as a novel branch of integrated lepidopteran pest management. Both treatments had a positive correlation to be considered as an effective control method. The toxicity of *Bta* increased twofold against irradiated larvae than un-irradiated ones. The pupation, adult emergence and survival were significantly decreased in simultaneous treatment than individual ones. Results of F_1 sterility study revealed that production of insect was significantly lowered in the simulations treatment than only IST. The durations of developmental stages were extended in various treatments. The sex ratio was more declined at the favor of male at ST as compared either with the control or the individual treatments. The findings indicated that IST based on F_1 sterility combined with *Bt* was common sense control methods than the use of IST individually and takes advantage of compatible control methods against lepidopteran pests.

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Author contributions
WS, RH and ThS contributed to the experimental and conceptualization, and RH and WS carried out the Bt bioassay analysis. WS and ThS conducted the irradiation treatment and biological studies, and RH and ThS carried out the statistical analysis. All authors contributed to the writing of manuscript and approved the final manuscript.

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

Declarations

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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