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### Ionizing Radiation Disinfestation Treatments against Pest Insects

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#### Abstract

Pesticides are often considered a suitable solution for controlling pests. However, the use of chemicals is very costly, and their residues have always the potential to pollute soil, air, and ground water and also pose significant risks to the natural ecosystems and nontarget organisms. Considering all these, irradiation could offer substantial and charming option for eliminating the export commodity fumigation uses for the undesirable effects of chemicals. Gamma rays, high-energy electrons, and X-rays are among the ionizing radiation sources utilized practically in sterile insect releasing programs using "self-contained" and "non-self-contained or panoramic" irradiators. When applying radiation sources, dosimetry should be adjusted to ensure quarantine security for large groups of insect pests. Because of growing concerns related to health problems and environmental pollutions, chemical sanitizing treatments are faced with a lot of regulatory restrictions, so irradiation reveals best choice for this purpose. The sterile insect technique (SIT) may have indispensable consideration for integrated pest management (IPM) of many important insect pests, including agricultural, veterinary, and medicinal importance. On the other hand, to overcome the obstacles of SIT treatments, genetic engineering techniques were supposed to ease the development of transgenic insects for sustainable tactics to control pest populations. Thus, genetic means should be an integral part of SIT treatments in controlling important pest populations.

**Keywords:** Ionizing radiation, dosimetry, sterile insect release, genetic sexing strain, F<sub>1</sub> sterility,



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#### 1. Introduction

Chemical pesticides have been the most widely used insect control methods, especially after the Second World War together with the invention of synthetic chemical pesticides. Pesticides are often considered a suitable solution for controlling pests. However, the use of chemicals is very costly and has polluted almost every part of our environment. Pesticide residues are found in soil, air, and ground water and they poses significant risks to the natural ecosystems and nontarget organisms.

There are two overriding problems facing insect controlling specialists. These concerns are the rapid development of resistance and environmental pollutions resulting from pesticide use. It was reported that irradiation could offer substantial and charming option for eliminating the export commodity fumigation uses for the undesirable effects of chemicals [1, 2]. In many countries, the direct control of stored product insects in wheat and wheat flour through radiation treatments is regarded as an approved method and would soon be approved for all grain products and other dry foods [3]. To this end, research is needed to continue for improving the methods. Although irradiation quarantine disinfestation treatment has been in progress for decades, it is not so common to use these tactics because radiation cannot kill the insects abruptly, and there are great concerns with regard to radiation applications among peoples. Due to the relationship with radioactivity and nuclear technology, consumers and industrial organizations have significant concerns about the radiation applications in food preservations, whereas even at the highest doses, radioactivity cannot be induced using these sources in food or insects exposed [4]. Accordingly, the development of radiation methods in controlling the agricultural products is so slow, and the adoption of these practices by the public and commercial organizations takes time. Informing the public awareness on the issue of the reliability of this method will enable more widespread use of these applications and will provide more acceptances by people. If safer and more secure products are obtained as a result of food irradiation and consumers are satisfied with the nutritional adequacy, their attitudes can be positive and they will buy the products without hesitation.

The superiority of irradiation in protecting agricultural products can be summarized as follows: it reduces product loss after harvesting. In terms of treating the products uniformly, it is more advantages over the fumigation treatments. It leaves no residues on the products and a best alternative to chemicals ensuring product quality standards in international trade [5]. It is also an important strategy for improving the hygienic quality of the agricultural products. The future inclination in quarantine measures against insect pests will mainly focus on the following issues: (1) determining specific doses for the insects resistant to radiation such as lepidopteran pests, (2) reducing radiation doses and abate treatment periods to maintain the product quality, (3) developing generic treatments lower than 400 Gy for important quarantine insects, and (4) developing information on value-added irradiated fresh products [6]. The standardized radiation treatments will facilitate safer trade between countries. The measures taken with radiation aim to prevent adult insect emergence. In this way, the risk of introducing exotic plant pest into new ecological areas during trade between countries can efficiently be prevented [6]. If there are eggs, larvae, and pupae in agricultural products, they are intended to be sterile. By examining numerous studies, these goals can be achieved with relatively lower doses for the pests belong to Diptera, Homoptera, and Coleoptera. Lepidopteran pests require higher doses than other groups. Radiation resistance of insects increase with advanced developmental stages. The tolerance of male insects is higher than that of females.

This review aims to provide information in presenting advances related to irradiation quarantine treatments against pest insects, assessing the worries in this field, discuss apprehensions with the applications, stressing the future trends, and explaining the mode of action of radiation on pest insects.

#### 2. Ionizing radiation sources

Ionizing radiation has been classified into X-rays,  $\gamma$ -rays,  $\alpha$ -rays,  $\beta$ -rays, and neutron radiation [7]. Nature and background are the main sources of ionizing radiation. Of these, cosmic radiation can be classified into various forms according to its origin, energy and type, and flux density of the particles. Tree main sources of cosmic rays are galactic cosmic radiation, solar cosmic radiation, and radiation from the earth's radiation belts (Van Allen belts) [8]. Gamma radiations have the possibility to ionize the atoms but not affect the nucleus, so they cannot induce radioactivity on irradiated materials [9]. Among the ionizing radiation sources, gamma rays, high-energy electrons, and X-rays are the types used practically in sterile insect releasing programs [10-12]. However,  $\alpha$  particles are not suitable for insect sterilization due to their high linear energy transfer and weak penetrability. On the other hand, neutrons are more effective in insect sterilizing, but their radioactivity induction in irradiated materials makes them impractical for sterile insect technique (SIT) programs [13-15]. It should be taken into consideration that for the fitness of insects, the acceptable level of energy for SIT applications is less than 5 MeV for Gamma rays (from 60Co and 137Cs) or X-rays and less than 10 MeV for electrons [4, 16-18]. Due to their similar relative biological effectiveness values, a different type of radiation source does not exert significant difference in their lethal effects on particular insects [4]. Cobalt-60 and cesium-137 radioisotopes are the most commonly used gamma radiation sources for SIT programs [8].

#### 3. Comparison of irradiators

Irradiators with several hundreds to thousands of Curies of a high-energy gamma or beta emitter are large self-shielded devices. The basic components of an irradiation unit (gamma-ray or electron) are composed of the following: (1) the control systems related to radiation source are referred as "irradiators," (2) a product transport system, and (3) a shielding for protecting human health and environment from radiation [4].

Two major types of irradiator are "self-contained" and "non-self-contained or panoramic." In the former, primary beam is entirely shielded during use and storage conditions. In the latter,

primary beam is not contained [19]. Irradiator design varies from small, which is suitable for radiation studies, to very large, which is convenient for hundreds of tons of product throughput daily. The activity level of the radiation source and the methods for the translocation of the products in the radiation field are the main differences between various irradiators [4].

Both cobalt and cesium are widely used as source rods in gamma irradiators [4]. Sterilization of insects is usually carried out with gamma rays from self-contained irradiators. In most self-contained irradiators, the position of irradiation is in the center of an annular array of long parallel pencils that include the encapsulated radiation source. Within this irradiation compartment, the doses are provided uniformly. Although self-contained irradiators provide a high-dose rate with a small irradiation volume (1–4 L), this design is suitable for small-scale programs that apply the SIT [4].

Panoramic irradiators are used efficiently for large-volume irradiation. In this design, the radiation source includes either several Co-60 rods lined up in a plane or a single rod that can be moved up and down into a wide chamber. Because gamma rays are emitted in all directions from isotopic sources, the high-energy utilization efficiency can be achieved through surrounding by insects, and irradiation can be applied to several containers at the same time [4]. Large-scale commercial irradiators are mostly not practical for practicing dosage-determining research due to differences between maximum and minimum absorbed doses received. Therefore, the determination of minimum absorbed dose required for an irradiation quarantine application is best performed using small irradiators [20].

Electrons and X-ray are the two modes of accelerator-generated radiation in electron and X-ray irradiators. The two characteristics of the principal electron-beam are the energy of particles (MeV), which affects the penetration of electrons, and the average current (in mA), which affects the rate of absorbed dose. Contrary to gamma rays, electron beams from accelerator-generated radiation are quite focused, and insects are continuously moved in conveyors through the beam. Due to the deeper penetration of X-rays compared to electrons, it allows the use of larger containers of insects for treatments [4].

Although sterilizing effects of electron and gamma rays are similar, the factors determining the source selection for SIT programs mostly depend on penetration, cost, product throughput, presence of experts, and safety factors [21]. Besides, gamma irradiators are normally cheaper and easier to run when compared with accelerators. However, due to their safety when switched off, the reliability and the public acceptance of electron accelerators are higher than gamma source [22-24]. The emission power of 100 kCi of Co-60 gamma-ray source is more or less equivalent to that of a 1.5-kW electron accelerator. The commercial accelerators have usually higher power capacities (5–10 MeV electrons), thus rendering them unsuitable for SIT applications. Although X-ray irradiators have the advantages over the gamma irradiators and accelerators, the effectiveness of transforming electrons into X-rays is nearly 7% for 5 MeV electrons. Thus, a great majority of the power is wasted while heating the converter [25]. When these conditions are all considered, gamma irradiators can be thought as mostly used in nearly all SIT programs [4].

#### 4. Dosimetry

Dosimetry is the radiation absorbed dose for sterilizing and is of major importance for programs that comprise the release of sterile insects [26]. Dosimeters are frequently used in producing sterile insects for such tasks as absorbed-dose mapping, process control, and qualification of the irradiator [4]. Some of them are convenient for routine use at SIT programs [27]. Insects receiving very low doses may not adequately be sterile, and those that absorb very high may be uncompetitive. In such cases, the effectiveness of the program that requires a greater number of sterile insects to be released may essentially be decreased [28]. In executing the analysis, variation in both dose-dependent sterility and competitiveness data are required at the same time. For the competitiveness data to be realistic, the tests should be performed in field cages or open plots [26]. Given the importance of dosimetry in SIT programs, selecting a convenient dosimetry system has a critical importance [26]. Methods for calibrating regular dosimetry systems and for determining radiation fields for insect sterilization are described in periodically updated ISO/ASTM standards [27, 29-31] and in IAEA technical reports [9]. Gray (Gy) is used as the absorbed dose unit, which is equivalent to a joule of absorbed energy per kilogram of sample [9]. Therefore, in newly planned programs, dosimetry system needs to be established for adequately measuring the absorbed dose and estimating the associated confidence interval [27].

#### 5. Doses achieve quarantine security

Irradiation is a quarantine treatment with the potential to disinfest a variety of fresh commodities of great number of quarantined pests. Many insect groups from the orders Diptera, Coleoptera, and Homoptera can be controlled with relatively low doses without damaging host plants of economic importance [20]. Other insects in Lepidoptera are controlled by moderate doses (0.2–0.3 kGy), which are tolerated by some major commodities, such as apples, cherries, and blueberries [20]. These doses need further evaluation using adequate numbers of insects to accomplish the degree of confidence required in quarantine treatments [20].

Moreover, because effective irradiation doses against most insects and mites do not affect the characteristics of commodities, this technology is ideal in developing "generic" treatments [32]. A generic quarantine treatment should provide quarantine security for large insect groups. For example, it can be applied to all pests belonging to Diptera, or to tephritid fruit flies in the genus *Bactrocera*. Before recommending generic treatments, effective irradiation doses should be evaluated in controlling the wide range of species belonging to a taxon [32] (Table 1).

Pest group	Objective	Dose (kGy)
Stored product moths	Adult sterilization	0.1–1
Stored product beetles	Adult sterilization	0.05–0.4
Pyralidae and Tortricidae	Late-pupa sterilization	0.2–0.3
Noctuidae and Tortricidae	Prevent adult emergence	0.1–0.3
Scarab beetles	Adult sterilization	0.05–0.15

 Table 1. Doses to achieve quarantine security for various pest insects [20]

#### 6. Advantages of irradiation over other postharvest treatments

The advantages of irradiation in controlling agricultural products can be outlined as follows: It is an effective and important tactic in controlling postharvest food losses. It is more advantageous compared to fumigation treatments due to its uniform penetration in the products and also time saving. It does not leave residues in commodities and a best alternative over chemical pesticides ensuring product quality standards in international trade. It is also an important strategy for improving the hygienic quality of the agricultural products [5]. The penetration power and the dose uniformity of the radiation treatment to treat products of different sizes and shapes and also to prevent the formation of resistance make the radiation treatments superior to chemicals [33]. Besides, radiation can reach pathogen organisms in areas of fruits not accessible to chemicals [34].

Because of growing concerns related to health problems and environmental pollutions, chemical sanitizing treatments are faced with a lot of regulatory restrictions. Thus, irradiation offers the most viable alternative for eliminating these concerns [5]. It was also reported that the minimum dose (150 Gy) required for disinfestation of fruit fly to satisfy quarantine regulations (0.15 kGy) does not adversely affect the physicochemical and nutritional value of most fruits and vegetables [35]. If the application is done properly, the efficacy of the irradiation process is guaranteed. It does not cause a significant amount of temperature increase during application; radiation does not leave residues. It is safe and removes concerns that may arise in terms of human health and environment. It is possible to apply for packaged products. However, some other disinfestation methods such as heat, cold, and fumigation treatments can be used in controlling pest insects in the commodities. For controlling pest species, irradiation treatments should be developed irrespective of commodity. Most products can have tolerance to irradiation at doses killing the pest; however, other methods cannot guarantee quality of the host commodities [4].

#### 7. A generic quarantine treatment

Introducing exotic pest insects through the improvement of international world trade in agricultural commodities becomes increasingly important problem day by day. This new

problem will cause extra costs for control programs and quarantine restrictions [36]. A generic quarantine treatment is one that provides quarantine security for a broad group of pests [37]. The International Consultative Group on Food Irradiation (ICGFI) was the first group to formalize a recommendation for a generic treatment. In 1986, based on irradiation data for many tephritid fruit fly species and a limited number of other insect pests, ICGFI proposed a dose of 150 Gy for fruit flies and 300 Gy for other insects [38]. Before generic treatments can be recommended, information is needed on effective irradiation doses for a wide range of insects within a taxon [36]. Data from all available insects are used in developing generic treatments because they serve as representatives for their respective groups [39]. According to a rule published in the United States in 2006, a dose of 150 Gy generic radiation was determined for all tephritid fruit flies and 400 Gy for all other insects, except for pupae and adults of lepidopteran pests, which require higher doses [40].

Some other applications such as heat, cold, and fumigation are used to disinfest host commodities before exporting them to pest free area. However, the treatment process other than irradiation requires balancing between the adverse effects and killing the pest insects to preserve commodity quality [41] since radiation treatments target pest insects without damaging the fruit or vegetable host [36]. For example, radiation prevents the temperature increase in commodities. International standard institutes approved that radiation is valid for all fruits and vegetables that are hosts for the given pests [42, 43].

Expanding the application spectrum of the generic irradiation treatments in the family or order level in other taxa would be practical, would easily promote international trade in agricultural products, and would supply an alternative treatment for infested commodities in cross-country transportation [44].

#### 8. Integrated pest management programs

The process of pest control is becoming more complex and requires new solutions in the course of time due to the emergence of new pest population, strict regulation in international trade, insecticide resistance, and residue problems. These new problems made it necessary to develop new and cleverly designed pest control techniques. Integrated pest management (IPM) is largely accepted as a powerful and environmentally sensitive method in managing pest insects that relies on a combination of commonsense practices [45]. In IPM strategies, comprehensive information on the life cycles of pests and their interaction with the environment is used. This method, in combination with available pest control tactics, is applied to manage pest population damage with the least possible hazard to people, property, and environment [45].

As a part of an area-wide integrated pest management (AW-IPM) approach, the sterile insect technique (SIT) is regarded as a vigorous control strategy for establishing pest free areas. The development of more competitive moths may improve the effectiveness of AW-IPM programs integrated with SIT technique [46, 47]. Species-specific nature and compatibility with existing control methods (biological control, mating disruption, cultural control, and use of biorational pesticides) make SIT an indispensable part of AW-IPM application and also make it superior

to other control methods [48-50]. There are a number of successful models in terms of integrating the SIT in AW-IPM programs against many important lepidopteran pests [51, 52].

Based on herein and other numerous literature, it may be said that SIT is a very convenient method as part of AW-IPM programs and can be further developed by decreasing the production costs, improving the effectiveness of released sterile moths and combining to other effective control tactics [53].

#### 9. Principles and practices of sterile insect technique

The idea that populations of economically important insect species might be controlled, managed, or eradicated through genetic manipulation was supposed by Knipling in 1930s. A similar concept was published independently by Serebrovsky [54]. In the late 1930s, Knipling recommended that if there could be a way to genetically sterilize male insects without affecting their ability to mate and competitiveness, then subsequent to their release and mating with wild females, the fertility of a target population could be reduced. The sterile insect technique is an environmentally innocuous and target-specific control tactic in suppressing the pest population [49]. With the development of modern genetic methods, this method will become a promising technique in the near future in controlling many important pest populations [55, 56].

The first applications were performed on the New World screwworm *Cochliomyia hominivorax* to evaluate this procedure [57]. The induction of sterility in this species by X-rays was the first small step on the way to the eradication of the serious livestock pest from Southern America and now from most countries of Central America [57]. This long-term and successful program has demonstrated that radiation-induced mutations can play an important role in developing environmentally acceptable, area-wide, and pest intervention strategies.

Although open to scientific criticism, the eradication process has been processed across the southern parts of the United States. With the help of this program, which began in Florida in 1957, the entire population of the pest is eradicated in the United States within a period of 10 years. Due to the reinfestations of migrating flies from neighboring Mexico, the program has been compromised, and the United States–Mexico joint program has become a necessity in 1972. With the success of the program, Mexico in 1991, Belize and Guatemala in 1994, and El Salvador in 1995 officially declared that they are free of screwworms. Because no flies have been detected since January 1995, Honduras was technically considered as free of screwworms. Eventually, the United States–Central America project proposed to maintain a sterile insect barrier at the Darien Gap in Panama starting in 1997. By the implementation of this program, billions of dollars was saved in livestock and wildlife loses [57]. Screwworm is an obligatory parasite of warm-blooded animals infesting livestock and mammals, including humans. Female flies lay their eggs on the wounded inflammatory region of the body. Larvae hatching from the eggs feed on the flesh. Because these flies were easy to rear, the program was composed of a small-scale wild adult population. The flies tend to mate only once the screw

worm was a good candidate for SIT program. These factors were optimum to achieve high sterile/fertile ratios fort this pest [58].

Although not as successful as the screwworm eradication program, SIT has been implemented for some other pest populations such as the tephritid fruit flies, including *Ceratitis capitata* Wiedemann, *Pectinophora gossypiella* Saunders, and *Cydia pomonella* L. in many parts of the world [59].

Pests of agricultural, veterinary, and medical importance can be specifically controlled using the SIT method, the integral component of AW-IPM. The sterile insect technique (SIT) is a specific control method that may be applied in the area-wide integrated pest management of insects. It is important to release only the sterile males to implement this method effectively [60]. As the next generation is to be established by wild females, the removal of wild males is essential for reducing the size of target population [61]. Infertility in the wild population can only be achieved with the help of sterile males. Thus, this method was initially named as the sterile-male method [62]. At the first application, both sexes were released in controlling the New World screwworm *C. hominivorax*. However, the benefit of this bisexual releases was determined to be limited for the Mediterranean fruit fly [63-65]. In such a design, released sterile males and females tend to mate with each other. This inclination reduces the mating potential of sterile males with wild females, and less sterility is introduced into the wild population. Only sterile male release reduces mass rearing costs for both production and postproduction stages. In the postproduction stage, considerable reductions can be achieved in the cost of workload, marking, irradiation, transport, release, and monitoring [66]. In many cases, releasing sterile females is not an easy process and brings about further negative effects. For example, females of fruit fly may cause extra damage in some fruits, females of biting flies result in reducing livestock meat production, and females of blood-sucking species may transmit disease [67]. However, it is not easy to make sex separation in such large populations. Therefore, to overcome this problem, it is obligatory to develop new specific strains. To date, Mendelian genetics, chromosome rearrangements, and specific mutations can successfully be used to develop new strains. When the sterile insect technique is compared with pathogenic biological entities and toxic chemicals, it is noninvasive. Therefore, the environmental risks of SIT application are exceptionally very low [68, 69]. This method is also compatible with the food chain in terms of integrating ecosystems with living but nonreproductive organisms. When considering all these situations, the hazard of the SIT to the environment is negligible.

There are some components that make the sterilization techniques successful [70], and the principles of sterility have not changed significantly since E. F. Knipling's formulation:

- 1. Mass rearing of target insects should be easy and applicable (rearing component).
- 2. Large numbers of the target insect should be possible to sterilize (treatment component).
- **3.** Following sterilization, fairly competitive insects should be released (competitiveness component).
- **4.** Release and distribution of sterile insects into fields should be cost effective (release component).

- 5. Before and after the release of the treated insects, population should be assessed accurately by using special tools (evaluation component).
- **6.** The treated area should be well isolated to prevent inseminated females from entering the field (reinfestation component).

# 10. Improvement of the sterile insect technique thorough genetic engineering technology

The sterile insect techniques (SIT) are considered as releasing sterile males in area-wide pest management. In this context, with the use of genetic methods, infertile matings were enhanced utilizing the release of mass-reared sterile insects [49, 71-73]. Therefore, by genetic means, new insect strains developed for improving SIT activity or avoiding potential adverse effects of such releases. Two categories of genetic methods for strain development are considered as conventional genetics and transgenesis [74, 75]. Using these methods, the development of an efficient and cost-effective SIT program would have a great importance in eliminating females from the released population. In this respect, the sterile insect technique may possibly be improved and extended using modern molecular tools. For example, SIT programs are improved by releasing unirradiated but instead homozygous insects with dominant lethal (RIDLs) constructs that are repressible during mass production [56, 76, 77].

A female-lethal version of RIDL, with insects homozygous for one or more femalespecific dominant lethal genetic constructs, has been created in *C. capitata* and offered for many other species [78]. This approach is also known as autocidal biological control [79]. The identification of alternative and more promiscuous transposable elements as *hermes*, *hobo*, *minos*, *mosI*, and *piggyBac* and novel gene delivery systems such as microinjection, electroporation, sonoporation, lipofection, and biolistics prompted studies on genetic manipulation of many insects of agricultural importance for various purposes [76, 80, 81].

The nature and timing of lethality is one of the most important potential advantages of genetic methods over radiation-based SIT programs. The transmission of transgenic SIT methods to insects of agricultural importance is now applicable through the development of sophisticated vectors incorporating the *piggyBac* transposable element [82, 83] and transformation markers based on improved green fluorescent protein (EGFP) variants [84, 85]. This technology was supposed to facilitate the development of transgenic insects for sustainable tactics to control pest populations or disease vectors [86, 87]. In addition, the use of systems for marking transgenic sperm in SIT programs is one of the other significant improvements for addressing the lack of efficient and reliable methods in field monitoring of insects. In SIT programs, producing male-only sexing strains for converting female insects into males thorough genetic manipulations in sex determining pathways can be another strategy [88, 89]. In the medfly, such a phenomenon has been shown to conditionally express a transgene that interferes with

the expression of female-specific *tra* gene expression. The resulting population was reported to comprise 95% males and 5% intersexes [89].

#### 11. Radiation-induced F<sub>1</sub> sterility in lepidopteran pests

If the parental generation of these insects was irradiated with substerilizing doses of gamma radiation, the degree of sterility would be higher than that of parental generation, and this circumstance is known as radiation-induced  $F_1$  sterility [15, 90]. Because the pest insects from Lepidoptera are radioresistant species, high doses are required to achieve complete sterility when compared to other pest insects from different orders [4, 91]. Despite continued for several generations, radiation-induced detrimental effects are most pronounced in the  $F_1$  generation. Inherited sterility is also referred as inherited partial sterility, partial sterility, delayed sterility, semisterility, and  $F_1$  sterility [52]. Mutagenic chemical substances (chemosterilants) can be used to induce sterility as an alternative to radiation, but due to human health and environmental concerns, chemicals are not preferable for obtaining sterilized mass-reared insects today [92, 93].

Inherited sterility has been shown for the first time on silkworm *Bombyx mori* (L.) [94]. Early investigations related to this topic were revised and discussed in terms of its pest control potential and genetic aspects [15, 90]. Experiments of the Proverb [95] showed that the  $F_1$  generation of insects was sterile when their parents irradiated with gamma radiation. This first application has opened up new horizons and given impetus to research on  $F_1$  sterility [96]. Knipling [97] and LaChance [90] recommended the use of  $F_1$  sterility as the potential component of area-wide integrated management of lepidopteran pests. The validity and efficacy of the method has been indicated on various pests in a number of laboratory studies [90]. However, a high dose of radiation adversely affects some important traits of the pest population as mating ability and longevity and causes reduction in the competitiveness of the sterile insects against the wild population [98]. This control tactic represents an environmentally friendly alternative and provides facilities for control of many important pest species. The superiority of this method over the completely sterile insect is discussed by many authors [52, 99-102].

For the high radio resistance in lepidopteran insects, the presence of possible DNA repair mechanisms and an inducible cell recovery system was proposed [91]. The radio-tolerant talent of these insects has also been attributed to the holokinetic nature of their chromosomes [103]. Radiation-induced sterility is generally a result of dominant lethal mutations (DLMs) in insects other than lepidopterans and is expressed during early cell proliferation in embryogenesis [52, 104]. However, the frequency of DLMs is much lower in Lepidoptera than that of other pest orders and is seen toward the end of embryonic development [105].

Since sterile  $F_1$  progeny are produced under field conditions, releasing partially sterile males with fully sterile females is more compatible with other tactics [106]. A significant amount of the unfertilized eggs or early embryonic mortality was observed for different lepidopteran pests in treated males mated with the females as in the case of *Manduca sexta* (L.), *Ephestia*  *kuehniella* Zeller, and *Spodoptera litura* (F.) [107-109]. It can be inferred that the most important cause of male sterility results from physiological impairments, including failed mating and inability to complete sperm transfer [52].

Males of Lepidoptera are more radio resistant than females. Several authors indicated that in different species of Lepidoptera, the sex ratio was biased toward the males [15, 108-111]. This difference is attributed to the gametes at the time of irradiation. Radiation is generally applied to mature pupa or newly emerged adults of Lepidoptera. Euprene sperm production is completed at the time of irradiation, and dividing cell reaches to interphase. However, the oocytes are stalled in metaphase I, and the process could not be completed up to the oviposition [112]. Thus, radiation disrupts the normal course of meiosis. The secondary harmful effect seen in the oocytes is the degradation of the cytoplasmic components. The treated oocytes have large amounts of cytoplasm than that of cytoplasm-free sperm, and this cytoplasm contains many components required for embryogenesis [52].

The higher sterility level in F<sub>1</sub> male progeny was attributed to three factors by Tothová and Marec [113]:

- **1.** Despite large inherited chromosomal breaks,  $F_1$  males continue to survive, and the frequency of the chromosome breaks indicates a positive correlation depending on increasing doses. However, this correlation is not seen in  $F_1$  females. The differences result from the large number of chromosomal breaks inherited by  $F_1$ , and higher radiation doses might cause increasing damage rate on sex chromosome (*Z*). F1 females might be affected of recessive lethal mutations, but not males.
- 2. Crossing-over process during spermatogenesis
- **3.** Radiation-induced deleterious effects on the fertility of  $F_1$  males

Genetic sexing system was suggested to introduce lethal mutations in the wild population firstly in *Bombix mori* by Strunnikov [114] and subsequently developed in Mediterranean flour moth *E. kuehniella* by Marec [115], Marec and Mirchi [116], and Marec [117]. Almost all  $F_1$  generations consist of male progeny due to the inheritance of one of the lethal mutations from their father when BL-2 males are mated to wild-type females. Females are hemizygous with regard to sex-linked recessive mutations (*sl-2 and sl-15*) and die during embryogenesis. For introducing lethal mutations into the wild population, balanced BL-2 males could be released directly into nature or could be reared in laboratory conditions to generate male mutant strains [108, 118]. The combination of  $F_1$  sterility with male-only colonies would be useful for reducing rearing costs and enhancing population suppression. Despite these advantages of genetic sexing system in  $F_1$  sterility applications against lepidopteran pests, lack of suitable markers for constructing mutant strains, difficulties in sex separation under mass-rearing conditions, and constantly checking requirements of mutant strains to keep its genetic structure through genetic recombination or colony contamination are some of the significant drawbacks that still need to be overcome [52, 119].

 $F_1$  sterility can effectively be combined with other control tactics, such as pheromone disruption [120-123], host plant resistance [122], and natural enemies [123]. The production of sterile  $F_1$ 

larvae should be considered as an opportunity for producing natural enemies and sterile moths in field conditions [124]. This would be an additional advantage ensured from this method. The extra eggs of sterile population will constitute additional host material for the egg parasitoids [111, 125]. These sterile eggs do not affect parasitoid preference adversely [126], and this tactic could also be a suitable way for combining SIT and augmentative release.

The benefits of the radiation-induced  $F_1$  sterility can be summarized as follows.

- Reduced egg hatch and highly sterile and predominant F<sub>1</sub> male progeny
- Lower doses are adequate to induce  $F_1$  sterility and hence to increase the quality and competitiveness of the released insects [15]
- Dispersal ability improvement following release [120]
- Increase in mating competitiveness [127]
- Improved sperm competitiveness [127]
- Sterile F<sub>1</sub> progeny production in the field
- Supplementing extra host material for the egg parasitoids [111, 125]
- For increasing the natural enemy population, F<sub>1</sub> eggs, larvae, and pupae of the pest insects can also be used as host [128]

## 12. International database on insect disinfestation and sterilization (IDIDAS)

The International Database on Insect Disinfestation and Sterilization (IDIDAS) is a data bank collecting the radiation doses applied to important pest arthropods, which are important in terms of veterinary, medicine, and agriculture. Data collection and share about radiation doses are the main purpose of this database for disinfesting and reproducing sterile pests by comparative analysis and quality check [129]. This data bank can be accessed from the website of IDIDAS [21].

#### 13. Conclusions and recommendations for future research

The future trends in controlling important pest population would predominantly be directed to biological control methods as SIT treatments. As an integrated part of area-wide pest management programs, the applications of SIT treatments will continue to increase and be desired by all sectors as farmers, commercial companies, and consumers. Cooperation and contributions of all stakeholders are essential to ensure effective implementation of these technologies. The development and the applicability of the proposed methods are required to be inexpensive and environmentally sensitive. For the mass rearing of biological control agents

and improving their transport facilities, various studies are carried out with great efforts. In this respect, with the utility of novel and innovative methods, the cost-effective augmentation of natural enemies in field conditions will be possible. The use of modern biotechnology and molecular methods for the manipulation of many insects of agricultural importance for increasing the competitiveness of released male-only population in the field, release of insects carrying a dominant lethal, and timing of lethality will contribute to radiation-induced sterility. Thus, sterile insect populations with highly competitive and desired properties can be achieved for protective purposes.

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