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Assessment of irradiation doses for sterility of vector mosquito and subsequent mating compatibility with wild females

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Abstract
The present studies were designed to develop long term sustainable strategies for the control of dengue vector in Pakistan. Mosquitoes were separated into males and females based on sexual dimorphism at the pupal stage. Ten transparent plastic bottle having 100 male pupae each were exposed to different radiation doses i.e. 20, 40, 60, 80 and 100 Gy by Cobalt 60 irradiation source. For *Culex* spp highest emergence of 90.50% was achieved with dose of 60 Gy and highest deformity of 25.25% was noted with 100 Gy. *Aedes* spp showed highest emergence (91.25%) and deformity (29.75%) with 60 Gy and 100 Gy, respectively. The mating frequency was acceptable at optimum doses of 40-60 Gy radiation. The radiation dose of 100 Gy yielded lowest adult emergence and highest deformity in the mosquito's species. The dose of 40-60 Gy was determined as the optimum dose for initiation male sterility required for SIT program.

Keywords: Cobalt 60, Irradiation doses, mating compatibility, sterility, wild females

1. Introduction
In Sterile Insect Technique (SIT) the laboratory reared sterile males are mass released into natural environment for the purpose of suppressing or eliminating the target pest by disturbing its progeny production [1, 2]. SIT has been used extensively and effectively to control various insect pest species [3]. In mosquito control, the Sterile Insect Technique dates back to 1960s when sterile *Aedes aegypti* males were released in Florida (USA), with the intention to reduce *Aedes* population [4]. This was followed by considerable studies on mosquito SIT [5]; pilot field trial in northern Sudan to determine the feasibility of SIT to control the African malaria vector *An. Arabiensis* [6]. Having potential for different species of mosquitoes, efforts for SIT tactics of *Aedes* species are also in progress at Insect Pest Control Laboratories (IPCL), Seibersdorf, IAEA, Vienna, Austria.
Benedict and Robinson (2003) explained the SIT use as a safe techniques comprising of mass production, releases and subsequent mating competitiveness with wild females [5]. The genetically modified mosquitoes have the potential of decreasing mosquito borne diseases transmission by releasing and establishing in the target sites. SIT is an environment friendly and species specific method of insect control in which large numbers of sterile insects are released. This is a useful insect control method against a range of agricultural insect pest and pests of public health importance [3].
Michelle *et al.* (2006) defined the three stages of SIT, i.e. mass production, sterilization and subsequent release of sterile insects into a target population in an area-wide integrated approach [7]. The released sterile males mate with wild females which no longer produce offspring and therefore the size of the target population is decreased. SIT has been proven to be a safe, effective and environment friendly approach to suppress and remove pest populations. The International Atomic Energy Agency (IAEA) has a long history of supporting SIT programs against tsetse flies, moths and fruit flies.
Alphey *et al.* (2010) reported SIT as an effective tool of vector control. They proposed that SIT is more valuable in the integrated multi-approaches control strategies and SIT may be very efficient that dramatically reduce the number of insects when the target vector density is decreased by other methods. However, the cost and benefits of SIT should be always assessed before planning any strategy for the control of mosquito populations, in the light of the

specific situation and local constrains [8]. Dumont and Chiroleu (2010) integrated chemical and mechanical control with SIT for mosquito control. They found that integrated approach including SIT control could be useful to control the wild mosquito population and thus lowered the risk of an epidemic [9].

Boyer *et al*., (2011) evaluated the mating ability of a local strain of *Aedes albopictus* using several batches of females and different cage sizes under laboratory conditions. Individual males inseminated 14 females at an average of 9.5 females/male, they were exposed to 20 females for 7 days. The average number of females inseminated/male was 5.3 when two virgin females were exposed to one male and replaced every day for 12 consecutive days, and 8.6 when 10 virgin females were exposed to one male and replaced every day for 14 consecutive days. It was suggested that the high number of females inseminated by one male and the duration of male activity may have strong implications in SIT for mosquitoes [10].

Baseline data on the working out effective radiation doses for male sterility and their subsequent mating compatibility with wild females are important towards the development of SIT of mosquitoes in Pakistan. This technique can be integrated with other control strategies which is important to know and was therefore, included in the current studies.

Keeping in view, the increasing trend of high mortalities from mosquito species vectoring dengue pathogens, there is a dire need to make short and long term planning for its management. The present studies were therefore, designed to develop long term sustainable strategies for the control of dengue vector in Pakistan. These studies will provide baseline entomological data which will help in better management of mosquitoes through the development of area wide integrated pest management strategies including planning for sterile insect technique in collaboration with international agencies. The current study was conducted with the objectives to determine optimum radiation doses for partial/complete sterility of dengue vectors.

2. Materials and methods

The present studies were conducted in the laboratory of Medical Entomology, Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan during the year 2012-13. Mosquitoes were differentiated and separated into males and females based on sexual dimorphism at the pupal stage. Conical flask was used initially for the separation of the pupae from the mix culture of the larval tubs. Care was taken to select the pupae of the same age. Then the male and female pupae were separated by using density principle, i.e. female pupae are large in size and thus denser which remained in the bottom of the flask as compared to the male pupae. The male pupae present on the surface of the container were effectively transferred using the standard mesh sieve mechanically. One thousand male pupae were separated in short period of 5 minutes. Ten transparent plastic bottle having 100 male pupae each was exposed to different radiation doses i.e. 20, 40, 60, 80 and 100 Gy (Gray) by Cobalt 60 irradiation source at NIFA, Peshawar. Each radiation dose was tested against the 4 sets comprising 100 pupae in the trials. The effect of each dose was worked out by recording the percent adult emergence (Number of adults emerged/Total Pupae*100) and testing their sterility by conducting the mating ability and subsequent progeny production ability. Deformities in the adults were also recorded as a result of the tested radiation doses. During male competitiveness test harmless dye was used for identification of sterile males and the wild females during the compatibility trials [11]. The data recorded were subjected to the analysis of variance technique and means were further separated through LSD test [12] using statistical package, Statistix 8.1 [13].

3. Results

The results in Table 1 reveal that irradiation dose significantly affected *Culex spp*. adult emergence and caused significantly variable adult deformities. Adult emergence was highest (90.50%) with irradation dose of Gy 60 and lowest (39.50%) with 100 Gy. Adult emergence was 72.50% in control. Generally, adult emergence increased with increase in radiation dose from 20 to 60 Gy, but decreased with the higher doses of 80 and 100 Gy. Adult deformities were significantly higher (25.25%) with irradiation dose of 100 Gy and lowest (1.75%) in control. Generally, adult deformities increased with increase in radiation dose.

Table 1: Effect of radiation dose on *Culex* spp. adult emergence and adult deformities during 2011-2012

Radiation dose (Gy)	Effect of radiation dose on		Overall Mean
	Adult emergence (%)	Adult deformities (%)	
0 (control)	72.50^{c}	1.75^{i}	37.12^{c}
20	59.00^{d}	3.25^{i}	31.12^{d}
40	81.50^{b}	4.25^{i}	42.87^{b}
60	90.50^{a}	7.00^{i}	48.75^{a}
80	48.75^{e}	17.75^{h}	33.25^{d}
100	39.50^{f}	25.25^{g}	32.37^{d}
Overall Mean	65.29^{a}	9.87^{b}	-

LSD value at 0.05% for dose = 3.75; LSD value at 0.05% for effect = 2.16

LSD value at 0.05% for interaction = 5.30

Means in columns/rows followed by similar letters are not significantly different (P= 0.05).

The results in Table 2 depict that irradiation dose significantly affected *Aedes spp*. adult emergence and caused significantly variable adult deformities. Adult emergence was significantly higher of 90.50 and 91.25% with irradation dose of Gy 40 and Gy 60, respectively. It was lowest (49.75%) with 100 Gy. Adult emergence was 82.0% in control. Generally, adult emergence increased with increase in radiation dose from 20 to 60 Gy, but decreased with the higher doses of 80 and 100 Gy. *Aedes spp*. adult deformities were significantly higher (29.75%) with irradiation dose of 100 Gy and lowest (2.25%) in control. Generally, adult deformities increased with increase in radiation dose.

Table 2: Effect of radiation dose on *Aedes* spp. adult emergence and adult deformities during 2011-2012

Radiation dose (Gy)	Effect of radiation dose on		Overall Mean
	Adult emergence (%)	Adult deformities (%)	
0 (control)	82.00^{b}	2.25^{h}	42.12^{b}
20	65.50^{c}	5.75^{gh}	35.62^{c}
40	90.50^{a}	6.25^{gh}	48.37^{a}
60	91.25^{a}	8.25^{g}	49.75^{b}
80	62.50^{c}	21.75^{f}	42.12^{b}
100	49.75^{d}	29.75^{e}	39.75^{b}
Overall Mean	73.58^{a}	12.33^{b}	-

LSD value at 0.05% for treatment = 3.18; LSD value at 0.05% for effect = 1.84

LSD value at 0.05% for interaction = 4.50

Means in columns/rows followed by similar letters are not significantly different (P= 0.05).

The results of the experiment on effect of irradiation on *Aedes spp*. fecunduty, egg viability and number of matings showed that irradiations dose significantly affected number of matings, fecundity in adult females and hatching of eggs in *Aedes* spp. (Table 3). It was found that number of matings in *Aedes spp* females were significantly higher in control (7.75 matings) and females treated with 40 Gy (7.00 matings). It was lowest in females (2.00 matings) treated with 80 Gy dose. Fecundity of *Aedes spp* females was significantly higher in control (126.0 eggs) and lowest (35.25 eggs) with radiation dose of 80 Gy. *Aedes* spp. egg hatching was significantly higher in control (87%) and lowest (0.25%) with radiation dose of 80 Gy. Generally, number of matings, fecundity and egg hathing in *Aedes* spp. decreased with increase in radiation dose.

Table 3: Effect of irradiation on *Aedes* spp. (No. of mating, fecundity and egg hatching under No-choice test during 2011-2012)

Radiation dose	No. of mating	Fecundity (No. of eggs/female)	Egg Hatching
0 (control)	7.75^{a}	126.0^{a}	87^{a}
40	7.00^{ab}	102.3^{b}	15.25^{b}
60	6.00^{b}	97.50^{b}	1.25^{c}
80	2.00^{c}	35.25^{c}	0.25^{c}
LSD value at 0.05%	1.31	11.58	5.35

Means in columns followed by different letters are significantly different (P= 0.05).

4. Discussion

Application of Sterile Insect Technique (SIT) entails the mass production, sterilization and subsequent release of sterile male insects in to a target population in an integrated area wide management strategy of mosquitoes. The released males inseminate wild females with sterile sperm. The females subsequently fail to produce viable offspring leading to an overall size reduction of the target population. Over the years sterilization by irradiation remains the most practical way of mosquito control. Effective optimum dose of radiation is required to produce potent males that are compatible for mating with wild females. We therefore, tested various radiation doses at the pupal stage of *Aedes* spp. and determination was made on effect of radiation doses on adult's emergence. The results of the effect of radiation on the biological parameters of *Aedes* spp. and subsequent potency of irradiated males with wild females are discussed below.

The experiment of irradiation of adult *Aedes albopictus* males and its subsequent mating with wild females under no choice test gave variable results, where number of mating decreased with increase in radiation doses. Lowest number of mating was recorded with 80 Gy radiation dose. *Aedes albopictus* fecundity decreased with increase in radiation doses. Maximum hatching was recorded in control. Michelle *et al.*, (2006) reported that irradiation of pupae, for all doses tested, had no effect on adult emergence and survival curves of males irradiated as pupae or adults were similar or even slightly higher than non-irradiated males [7]. This may be due to differences in temperature or improper use of sterilization indicating no effect on adult emergence. In our results, 60 Gy dose was found optimum for the desired number of mating, no or very low fecundity and hatching and thus may be considered in the Sterile Insect Techniques (SIT) of *Aedes albopictus*. Michelle *et al*., (2009) reported similar requirements for SIT of mosquitoes, they emphasized on the certain level of stability between sterile males and wild females. The sterile males should be compatible in mating with wild female under field conditions [14].

The use of SIT as a mosquitoes control strategy has been reported by some earlier researchers. Benedict and Robinson (2003) detailed the use SIT as a safe technique including of accumulation production, releases and subsequent mating aggressiveness with wild females [5]. Dyck *et al*., (2005) reported that SIT is an insect pest control method with a great success against agricultural insect pests [3]. Michelle *et al.*, (2006) stated that SIT has confirmed to be a safe, effective and environmentally sound method to suppress, eradicate or contain pest populations [7]. Bellini *et al.,* (2007) declared *Aedes Albopictus* species as more suitable for application of the SIT because of its urban-related distribution, low active dispersal potential and low population density [15]. Alphey *et al*., (2010) suggested that SIT is more useful in the situation of integrated multi-approaches control strategies and it may be very effective in reducing the number of insects [8].

5. Conclusion

The radiation dose of 100 Gy yielded lowest adult emergence and highest deformity in the mosquito's species. The dose of 40-60 Gy was determined as the optimum dose for initiation male sterility required for SIT program. The sterile males at these optimum doses were found potent and compatible in mating with the wild females. This information can be utilized as baseline data for launching SIT program of *Aedes albopictus* on pilot scale in Pakistan.

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7. Competing Interest

The authors declare that they have no competing interests.

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