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Effect of gamma radiation on life history traits of *Aedes aegypti* (L.)

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ABSTRACT

Aedes aegypti is an important vector for Dengue and Dengue hemorrhagic fever. Considering its medical importance and its relevance as a model system, this study was undertaken to evaluate the impact of different doses of gamma radiation for three generations of *A. aegypti*. Two to three days old virgin males of *A. aegypti* were irradiated with 15 doses of gamma radiation, ranging from 1 to 50 Gy and were immediately mass mated with the same aged virgin females. Observations were made for changes on their life history traits, particularly fecundity, hatchability, adult emergence, sex ratio and longevity, for three generations. Adult males exposed 30, 35, 40, 45 and 50 Gy doses showed a significant decrease in fecundity in F_0 generations. While hatchability was observed to have decreased with increasing radiation doses from 3 Gy onwards in the F_1 generation, samples irradiated with 30, 35, 40, 45 and 50 Gy maintained significant decline in hatchability in their succeeding generations, F_2 and F_3 also. Similarly, a decline was observed in adult emergence from 3 Gy onwards in all three generations. A male favoring sex ratio distortion was observed at the doses of 35, 40, 45 and 50 Gy in all three generations. Following exposure to 4 Gy, parental males and the resultant progeny showed increased longevity by 10.56 and 8.66 days respectively. Similarly, the F_1 generations of samples irradiated with 30, 35 and 40 Gy exhibited an increase in longevity by 7.16, 7.44 and 6.64 days respectively. Dose response curve for fertility among the three generations was drawn and presented. The effect of radiological exposure on the life history traits of *A. aegypti* varies with dose for the three generations studied. These results have potential implications in mutational studies and risk assessment and also contribute to a better understanding towards employment of the sterile insect technique in *A. aegypti*, plausibly paving the way to an effective mosquito genetic control program.

1. Introduction

The effects of ionizing radiation on various units of the ecosystem have always been of immense scientific interest. Numerous studies have documented the adverse effects of these radiations on human beings. However, studies of its effects on non-human biota are relatively fewer (EMRAS (Environmental Modeling for RAdiation Safety), 2007; Moller and Mousseau, 2013). While there are studies that suggest that ionizing radiations induce an adaptive or hormetic response in living organisms, especially at low doses, most surveys have revealed its detrimental attributes. Understanding the bigger picture involves discerning the impact of ionizing radiations on all

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components of the ecosystem (UNSCEAR, 2011). Hence, studying the effect of ionizing radiation on other, non-human yet significant members of the ecosystem is necessary. The interaction between radiation and a wide range of biological species is not very well understood because of their great variation in life cycles, life spans, and exposure pathways (Singhal et al., 2009).

Aedes aegypti is an excellent model organism; because of its ready adaptability to laboratory culture and short life span with high reproductive potential, this species has been used as a test animal for many physiological, developmental, biochemical and behavioral studies (Craig and Hickey, 1967; Clemons et al., 2010). This species is more popularly known as the primary vector for Dengue fever/Dengue hemorrhagic fever (Ahmad et al., 2007). Numerous vector control measures have been initiated to curb its proliferation. Popularly practiced insecticide based control of insect vectors/mosquitoes faces two important challenges, namely — the development of resistance to insecticides and the environmental pollution caused due to the constant application of insecticides. It is desirable to have alternate strategies which do not involve resistance among the vectors and genetic control or autocidal control is one such method (Shetty, 1997). The Sterile Insect Technique (SIT) is one of the genetic control methods, which involves the release of sterile males into the environment in an attempt to check its proliferation (Knipling, 1967). Considering, the relevance of *A. aegypti* as a model system and its medical importance as a vector species, radiation studies on this organism would be highly beneficial. Understanding the effect of ionizing radiation on this species is not only necessary from an ecological context but also from an anthropical and prophylactic perspective (Hart, 1980). A previous study on gamma irradiation of adult males of *A. aegypti* has shown reduced productivity or offspring at a high dose of 50 Gy (Rodriguez, 1977).This study however has not delved into the detailed impact of radiation on the life history traits of the said species. Ionizing radiation induced lethal mutations can be expressed at any stage in the insect's life cycle (LaChance and Crystal, 1965). Our prime interest is to understand the variation in effects produced by different doses of radiation on the life history traits of the said vector species. A comprehensive study such as the present one, will give better insights to the effects produced by the different doses, on the physiology of the organism and whether it is carried into the next generation. This could also help identify an optimal dose for radiation induced male sterility in *A. aegypti*, thus providing useful insights into a genetic control program.

Apart from radiation, various environmental factors have also been shown to induce variations in the life-history traits of several mosquito species (Lyimo et al., 1992; Phelan and Rotiberg, 2013). As with such studies, it is necessary to evaluate the persistence of the induced effects of different doses of radiation across multiple generations, in order to achieve a clear perspective of the consequences of exposure of a population, to ionizing radiation. Within this context, this paper presents a comprehensive study examining the effects of fifteen doses of gamma radiation, ranging from 1 to 50 Gy, on the life history traits of *A. aegypti*, with specific focus on fecundity, hatchability, adult emergence, sex ratio and longevity for three generations.

2. Materials and methods

2.1. Mosquito culture

A. aegypti used in the present study was originally collected from J.P. Nagar, Bangalore, India and has been colonized since 2011. The larvae and adults were reared in an insectary maintained at 25 ± 1 °C and 75 ± 5% relative humidity under 14 h photoperiod (Shetty, 1983). Adults were maintained in 21 cm cubic cages of iron frames covered by cotton net cloth and fed on 10% sucrose solution in a jar with a cotton wick. Females were provided with the blood meal from restrained mice upon maturation. A plastic cup (7.62 cm diameter) containing clean water lined with filter paper was placed inside the cage for oviposition. The hatched larvae were transferred to enamel trays and reared. Powdered mixture of yeast tablets (Geo Pharmaceuticals, Bangalore) and dog biscuits (Pedigree, Mars Industries, Hyderabad) were provided as larval diet. All mosquitoes used in the experiments were reared at a density of ~600 larvae per tray (25 × 30 cm) containing ±2 l of water (water depth 2 cm).

2.2. Pure line (PL) synthesis

In order to eliminate the recessive lethal or sterile genes, in the laboratory populations, if any, about fifty gravid females were randomly selected from the population cages and were isolated individually in separate vials for egg laying, following the procedure of Shetty (1983). Females producing highest number of viable eggs were selected for further inbreeding. The process of selection and inbreeding was continued for seven generations in order to increase the fertility rate. Stocks with high fertility rate were used for further experimental assays.

2.3. Irradiation study

Experiments were performed in batches for different doses and a control. Overall a total of 1500, 2–3 days old unmated PL males were isolated and irradiated with different doses of gamma radiation from ^{60}Co (Theratron 780-C Telecobalt Unit, AECL, Ontario, Canada) source with a dose rate of 253.56 cGy/min, at the Kidwai Memorial Institute of Oncology, Bangalore. Mosquitoes were irradiated in small 2.5 cm depth plastic square boxes (5 × 4 cm) covered with fine net cap. A dosimetry was used to quantify the dose received by the irradiated insects and it was confirmed that all the doses delivered lay within a 5% error range. Doses of 1, 2, 3, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45 and 50 Gy were chosen for the study. Each batch consisted of 100 adult mosquitoes receiving a specific dose of the radiation. The irradiated males (n = 100) were transferred into five different cages (20 × 20 × 20 cm) and immediately mass mated with virgin females of the same age (n = 100), such that each cage contained 20 irradiated males and 20 virgin females. Each cage was maintained separately to serve as replicates, such that the progeny from each of the five different