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Effect of Gamma Radiation on Survival and Fertility of Male *Anopheles stephensi* Liston, Irradiated as Pharate Adults

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Keywords

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Abstract

Effect of radiations on the survival and fertility of male *Anopheles stephensi* Liston was evaluated in the laboratory with 70 and 100 Gy radiation dose. Irradiation of pharate adults with 100 Gy dose resulted in reduced emergence and longevity as compared to control (0 Gy) and 70 Gy dose. Fecundity and fertility of female *An. stephensi* mated with male irradiated as pharate adults was determined upto four blood meals. Significant decline was observed in fecundity after each blood meal at 100 Gy as compared to 0 Gy and 70 Gy irradiation. Similarly, the fertility was also decreased after each blood meal at 100 Gy irradiation. The present study indicates the suitability of 100 Gy dose for irradiation of pharate adult stage of mosquito as a part of mosquito management.

1. Introduction

Across the developing world, the public health burden due to vector borne diseases continues to multiply, as current control measures fail to cope with the present scenario. There is an urgent need to identify improved control strategies that could remain effective even in the face of growing insecticide and drug resistance [1]. Eco-compatible biological based control approaches are gaining tremendous attention to tackle mosquito problem at present [2]. One such approach is the Sterile Insect Technique (SIT), which relies on the sterilization of insects, either by chemosterilization [3-5], irradiation [6] or modern biotechnological approaches [7-8]. The biotechnological approaches based on transgenic organisms are promising but are at an early stage of development and no legal framework yet exists to facilitate the introduction of such organisms in the wild [9-10]. Sterilization of mosquitoes by irradiation or chemosterilants has not been researched extensively for about last thirty years [11]. Promising results were obtained with chemosterilants in terms of the level of sterility induced and competitiveness but these have disadvantage of being mutagenic agents. Hence, they present a potential hazard to humans during the treatment process and non-target organisms if residues persist in the released individuals [5].

Malaria is the most important insect transmitted disease affecting 300-500 million people worldwide each year mainly in Africa and south of Sahara. About 2.7 million people are killed either with malaria alone or in combination with other diseases, and over 2400 million remain at risk. In India around 2 million malaria cases at average are reported annually, but the real picture is grossly underestimated due to various logistic lacunae [1]. In India, out of 58 *Anopheles* described, six namely *An. culicifacies, An. dirus, An. fluviatilis, An. minimus, An. sundaicus* and *An. stephensi* have been implicated to be main malaria vectors [12]. *An. stephensi*, a sub-tropical species is an important urban malaria vector in India, Pakistan and Iran [13]. Many international efforts [14-15] have been launched using SIT despite of scepticism in parts of India's scientific community, with the aim of eradicating *An. stephensi*. SIT relies on the fact that female mosquitoes mate only once and if this single mating occurs with sterilized males, it results in eggs that do not hatch. So, releasing sterilized males in numbers far greater than those of the wild male population could eradicate the targeted vector mosquitoes provided the area is not re-infested by mosquitoes from the surrounding region. The SIT projects have been developed for *An. arabiensis* Patton, the malaria vector in Reunion Island and along the Nile in Sudan [15], and might be appropriate for suppression of ecologically isolated urban island populations of *Anopheles* in India and Nigeria [16]. Control of mosquito vectors using SIT can be achieved more effectively in the small towns or islands, where only one or two kinds of vectors are present [14]. Although the long-term efficacy of SIT in eradicating mosquitoes has not been demonstrated till now, however the success in controlling screw worm, tsetse and mediterranean fruit-flies utilising SIT [17-18] can play a leading role in deploying this technology in malaria control.

The present investigation was aimed to understand the effects of gamma radiation on longevity, emergence, fecundity (oviposition) and fertility (egg laying potential) of *An. stephensi* in order to emphasize the potential of this eco-friendly method in the control of malaria vector in Indian subcontinent.

2. Materials and Methods

Mosquito culture for experimental studies was obtained from the laboratory colony of *An. Stephensi*, maintained at the NIMR (National Institute of Malaria Research), Delhi, India. The culture was maintained at optimum environment conditions (27 ± 1°C temperature and 75 ± 5% relative humidity). Irradiation of insects was performed in the Radiobiological unit of INMAS (Institute of Nuclear Medicine and Allied Sciences), Delhi using the ^{60}Co source (Gamma - 5000 irradiator, BRIT, BARC, Trombay). The dose rate of radiation was approximately 2.37 KGy/h. Seventy and 100 Gy radiation doses were selected on the basis of earlier work on *Anopheles* sp pupal stage [3, 11].

Male pharate adults (pupae 24 h before emergence) were sexed microscopically, placed on wet cotton in petriplates and transported to radiation source in the ice chest for irradiation dose [11]. After irradiation male pupae were kept in 30 cm^3 cages. The mosquitoes that emerged successfully were counted whereas semi-emerged adults and dead adults were scored as non-emerged. The emerged adults were fed on 10% glucose solution in soaked cotton swabs. Longevity and mortality record of the adults was maintained daily till all the adults died.

For assessment of fecundity and fertility irradiated male and non-irradiated female pharate adults were kept together in 30 cm^3 cages after emergence and were provided with 10% glucose solution soaked in cotton swabs along with raisins. Mates were introduced into experimental cages in a 1:1 ratio. Each treatment was repeated at least thrice and 20 males and females each were taken for each replicate. For each cage, one egg bowl filled with water and lined with wet filter paper was offered two days after blood meal. The eggs laid by female mosquito were removed daily and kept in larger trays for hatching after counting [3]. Eggs hatchability was checked by counting first instar larvae (L_1) to monitor the fertility. Fecundity was calculated by dividing the number of eggs laid after blood meal by the number of female alive.

Data analysis: Comparison for fecundity and fertility among 0 Gy, 70 Gy and 100 Gy irradiation dosages and blood meals were carried out using ANOVA. Similarly longevity and emergence percentage among the three dosages were compared using ANOVA. The values in the table are expressed as mean± standard error of mean.

3. Results and Discussion

The fecundity of female mated with 100 Gy irradiated male observed after first, second, third and fourth blood meal was 35.6± 3.7, 29.8± 3.9, 24.7± 4.7 and 30.7± 4.6 (mean± SEM) respectively (table-1). The variation in fecundity among the four blood meals at 100 Gy and 70 Gy was found non significant (p= 0.404, df= 3, F= 1.098 and p= 0.066, df= 3, F= 3.585). On the other hand the fecundity after every blood meal was significantly reduced when irradiated with 100 Gy as compared to control (0 Gy) and 70 Gy (p≤ 0.026, F ≥ 7.113).

Table: 1. Effect of gamma radiation on fecundity of female *An. stephensi* mated with male irradiated as pharate adults

Dose (Gy)	After 1st blood meal (Mean± SEM)	After 2nd blood meal (Mean± SEM)	After 3rd blood meal (Mean± SEM)	After 4th blood meal (Mean± SEM)	F -stat
0 (95% CI)	56.9 ± 4.9 (35.9- 77.8)	55.0 ± 5.7 (30.5- 79.5)	55.6 ± 4.6 (35.8- 75.4)	64.2 ± 1.6 (57.0- 71.4)	p= 0.479, F= 0.906
70 (95% CI)	46.1 ± 3.3 (32.0- 60.1)	32.3 ± 2.8 (20.0- 44.6)	44.7 ± 3.5 (29.5- 59.8)	32.7 ± 5.5 (8.8-56.5)	p= 0.066, F= 3.585
100 (95% CI)	35.6 ± 3.7 (19.7- 51.4)	29.8 ± 3.9 (13.1- 46.5)	24.7± 4.7 (4.3- 45.1)	30.7 ± 4.6 (10.9- 50.5)	p= 0.404, F= 1.098
F-stat	p= 0.026, F=7.113	p= 0.011, F=10.363	p= 0.006, F=13.145	p= 0.002, F=19.404	

SEM = standard error of mean; F = Fisher's coefficient

Male longevity (days) recorded at 100 Gy was 11.8 ± 0.264, which was significantly less than that recorded in control and 70 Gy (p= 0.024, df= 2, F= 7.447) (fig 1). Similarly, male emergence (%) was also reduced significantly after 100 Gy as compared to control and 70 Gy irradiation (p= 0.009, df= 2, F= 11.259) (fig-2).

Fig 1: Male longevity in *An. stephensi* irradiated as pharate adults

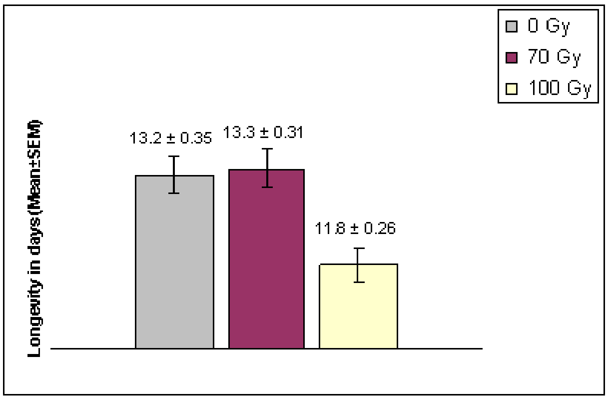
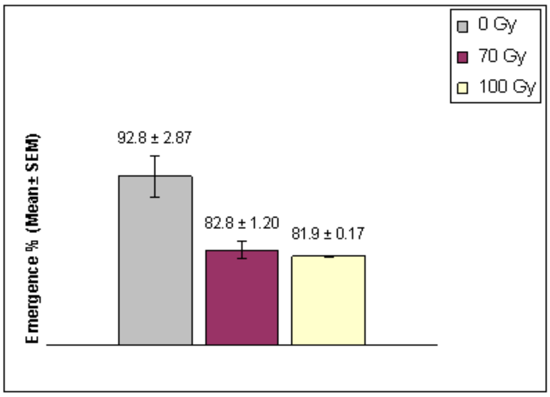


Fig 2: Male emergence in *An. stephensi* irradiated as pharate adult



The fertility of female mated with 100 Gy irradiated male decreased significantly after each blood meal (p< 0.0001, df= 2, F= 79.188 for first, p< 0.0001, df= 2, F= 97.863 for second, p< 0.0001, df= 2, F= 126.42 for third, p< 0.0001, df= 2, F= 136.83 for fourth blood meals respectively) as compared to control and 70 Gy irradiation (table-2). Similarly, fertility after fourth blood meals at 70 Gy was less as compare to other three blood meal (p= 0.026, df= 3, F= 5.312). However no difference was observed in fertility among four blood meals at control and 100 Gy irradiation (p= 0.865, df = 3, F= 0.241 and p= 0. 086, df= 3, F= 3.147).

Table: 2. Effect of gamma radiation on fertility of *An. stephensi* when normal females were mated with males irradiated as pharate adults

Dose (Gy)	After 1st blood meal (Mean± SEM)	After 2nd blood meal (Mean± SEM)	After 3rd blood meal (Mean± SEM)	After 4th blood meal (Mean± SEM)	F -stat
0 (95% CI)	74.4± 4.4 (55.64- 93.82)	72.4± 4.6 (52.72- 92.01)	69.6± 4.2 (51.31- 87.81)	72.6± 3.9 (55.41-89.79)	p= 0.865, F= 0.241
70 (95% CI)	22.9± 4.7 (2.57- 43.16)	24.6± 3.2 (10.77- 38.36)	9.1± 3.6 (-6.46- 24.66)	6.96± 4.1 (10.81- 24.75)	p= 0.026, F= 5.312
100 (95% CI)	7.5± 2.3 (-2.29- 12.22)	6.90± 2.0 (-1.75- 15.55)	1.9± 1.3 (-3.72- 7.52)	1.9± 1.0 (-2.61- 6.35)	p= 0.086, F= 3.147
F-stat	P< 0.000, F= 79.188	P< 0.000, F= 97.863	P< 0.000, F= 126.420	P< 0.000, F=136.830	

SEM = standard error of mean; F = Fisher's coefficient

Mosquito control using SIT methods have received very little attention however various SIT based control programmes at small scale have produced good results. In *Aedes aegypti* radiations have affected the normal insemination and fecundity by inhibiting normal accessory gland activity [19]. The decline in fecundity potential of female mosquito is important as less number of egg are laid, which ultimately decrease the vector density in a particular area of interest. Our results were nearly similar to those obtained elsewhere [11] in which pupal irradiation produced 83.4 % sterility at 70 Gy and 98.6 % sterility at 100 Gy in *An. arabiensis*. In *An. Stephensi*, Sharma *et al.* (1978)[3] reported that male pupal irradiation with 80 Gy induced 97.2% male sterility whereas, irradiation of 120 Gy produced 99.1% sterility and reduced fitness of males for mating and survival.

Over the past few years, ionizing radiations have been proved to be a reliable way to induce sterility in variety of insects [6] which strongly favour the exploration and development of sterilization protocols for possible use of SIT against malaria vectors. Most of the earlier work on *Anopheles* irradiation has been focused on the pupal stage [21, 22, 20, 3]. The irradiation of pupae is preferably performed on older (> 15 h) pupae since irradiation of young pupae may result in reduced emergence [21, 23] and shorter survival of the adult [24].

Helinski and co workers (2006)[11] have reported that emergence and survival rates of *An. arabiensis* mosquitoes irradiated as 22-26 h old pupae were similar or reduced as compared to unirradiated control mosquitoes of same species. Most of the studies carried out on mosquito longevity reveal the decrease in male longevity after irradiation at doses of 100-130 Gy [21] and 80 Gy [3]. Likewise, we have observed a considerable decline in both emergence and survival rates. In contrast, a few studies indicate the non significant increase in the longevity of mosquito after irradiation [25]. Irradiations are mainly intended to target the germ cells of subject organism but the irradiation process is highly non-specific and sometime somatic damage may occur. One of the commonest effects of somatic damage seen after irradiation is reduced longevity [21, 23].

4. Conclusion

While discussing SIT for mosquito control programmes, the irradiation is most favourite option for sterilization. The emergence, longevity was decreased significantly, which indicate the possibility to irradiate the pupal stage of mosquito. Further the fecundity and fertility was also reduced considerably using radiation at 100 Gy. The present study suggests the use of a dose range 70-100 Gy to sterilize *An. stephensi* pharate adults in integrated malaria control programmes. However, extensive study results produced in field conditions are necessary to determine the optimum dose and suitable developmental stage for irradiation.

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