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Abdalla Ibrahim Abdalla
Assistant Professor, Faculty of Health and Environmental Sciences, University of Gezira, Sudan

Mutaman AA Kehail
Associate Professor, Biosciences and Biotechnology, Faculty of Engineering and Technology, University of Gezira, Sudan

Nasir A Ibrahim
Assistant Professor, Faculty of Sciences, University of Hail, KSA

Correspondence
Abdalla Ibrahim Abdalla
Assistant Professor, Faculty of Health and Environmental Sciences, University of Gezira, Sudan

Effect of X–ray on the fertility, fecundity and sterility of female *Anopheles arabiensis* mosquito (Diptera: Culicidae) in Gezira State, Sudan

Abdalla Ibrahim Abdalla, Mutaman AA Kehail and Nasir A Ibrahim

Abstract
In Gezira State, Sudan, the use of the insecticides for control of agricultural insect pests and mosquitoes, has led to environmental pollution, pest resurgence, pest resistance and appearance of secondary pests. The aim of this work was to study effect of X–ray radiation on adults and larvae of *Anopheles arabiensis* mosquitoes, specifically the reproductive periods and fecundity. Some larvae and adults of *A. arabiensis* were irradiated with different doses of X-ray. The insects were then reared and the pre oviposition period, daily number of eggs deposited, the oviposition period, the post oviposition period, the adult longevity and the average number of eggs per female were recorded. The results revealed that, the average fecundity was 110.34 and 93.2 (eggs per female) for *A. arabiensis* females mated with irradiated males at their adult stage and at their larval stage, respectively. In the control group it was 29.1-147.3 eggs per female. The average sterility was 75.59% and 41.17% for *A. arabiensis* females mated with irradiated males at their adult stage and at their larval stage, while it was 2.35% and 0.10% in the control group, respectively. This study recommends using X-ray techniques to control *Anopheles* mosquito's population since it decreased the fecundity and induced sterility in males.

Keywords: X–ray, Fertility, Fecundity, Sterility, *Anopheles arabiensis* Mosquito, Gezira State, Sudan

1. Introduction

Anopheles mosquito have a world-wide distribution, occurring not only in tropical areas but also in temperate regions and they are absent from the extreme northern parts of the temperate zones [1]. The order Diptera is of prime importance in medical entomology, containing as it does blood sucking forms, disease vectors and various nuisances. Mosquitoes are small insects (about 3-6 mm long) with long legs, a globular head, laterally compressed thorax and long cylindrical abdomen. The wings are rather long and narrow, and are carried flat over the back in repose. The mouthparts used for piercing and sucking. The antennae are pilose in the female and plumose in the male. Male and female will live for several weeks, feeding repeatedly under natural conditions. Breeding places are always in water. Larva takes one week to complete development in tropical regions, but it over winter as larvae in temperate areas. The fourth instar larva moults to the pupal stage, from which the adult mosquito emerges after a few days. When foraging, a blood -thirsty female mosquito flies upwind searching for the scent trail of an attractive host [2].

The Gezira irrigated area (over 2 million feddans), lies between latitude 13N and 15N in the central part of the Sudan. The area has a hot-dry summer from April to June with an average daily temperature of 32 ^{0}C and RH of 20%, and a cool-dry winter from December to March, with an average daily temperature of 22 ^{0}C and RH of 30%. Average rainfall is about 225 mm/annum falling between July and September. The habitat in the Gezira continued to be more suitable for breeding of mosquitoes all around the year. Mosquitoes were suggested to be involved in some diseases other than the ordinary known ones and some other unknown diseases. Therefore, the control of mosquitoes must mean more than to control malaria and nuisances. Mosquitoes are subjected to intensive insecticidal pressure as a result of health and agricultural pests control operations. The genus *Anopheles* includes over 400 species. *A. gambiae* Giles complex is among the major vectors of malaria and filariasis. *A. arabiensis* Patton is the principal malaria vector in the Gezira area [3]. The ecology and behavior of mosquitoes were well reviewed by Service [4].

In Gezira State, Sudan, insecticides were used for many years for controlling mosquitoes and other agricultural pests; insecticides are effective and can be easily applied. The use of the insecticides for control of agricultural insect pests and mosquitoes, has contributed to the environmental pollution, pest resurgence, pest resistance and appearance of secondary pests. With the growing understanding of problems solving from insecticidal pest control, notable progress had been achieved in the field of insect pest control through genetic manipulations of insect populations. The gradual accumulation of these insecticides leads to many diseases in man and animals, in addition to that, many strains of mosquitoes developed resistance to these insecticides and the problem of mosquitoes still remains [5].

Genetic engineering for mosquito control appears to be a new method with a very simple idea, that is, the possibilities of inducing sterility on insect population through the use of sexually sterile males [6]. Although the theory was early suggested, it was not until the late sixties that it was rediscovered and demonstrated in the tsetse fly and in mosquitoes [7] and in the housefly [8]. In a recent comparative theoretical evaluation of the various genetic methods, it was concluded that, the efficiency of translocation strains in suppressing populations was extremely high relative to most other genetic mechanisms [9]. Several translocation strains have been successfully produced in a number of insect pests specially mosquitoes. Apparently a few of them were reported in *Anopheles albimanus* [10]; *Anopheles gambiae* [11, 7]. *Anopheles stephensi* [12] and *Anopheles culicifacies* [13]. It was also concluded that, successful genetic control will be difficult on a large scale, but possible in isolated population [14].

2. Research Objective

To study the effect of X–ray on the fecundity, fertility and sterility of female *A. arabiensis* (collected from Gezira State, Sudan) irradiated at their larval and adult stages

3. Materials and Methods

3.1 Sampling of Larvae

Sampling of larvae was carried out in three locations within Gezira State, Sudan during 2009 and 2010. In each location, the mosquito larvae were collected by means of metal dish (15 cm in diameter and 10 cm deep) put in plastic bucket (8 liters). The larvae of *A. arabiensis* mosquito collected from each location were kept in a metal dish (20 cm in diameter and 10 cm deep) which supplied with dry yeast (0.5 mg/ liter water) as a food for rearing. The adults were reared inside a rearing cage (60 x 60 x 60 cm) that covered with white sheet of cloth described by Stewart [15].

The source of blood meal for female mosquitoes was a pigeon trapped in separate small cage within the rearing cage. The axial feathers of the pigeon were removed, wings were tied and the bird was trapped to its cage every night during the period of females feeding. For the male mosquitoes the source of energy was 10% sugar solution provided in a cotton wick put in an open Petri-dish. A trough containing tap water (20 cm in diameter and 10 cm deep) was provided to each adult's cage for oviposition.

3.2 Submission of Larvae and Adults to x-ray Radiation:

For the larvae, a number of (220 -330) of *A. arabiensis* mosquitoes were put in eleven glass beakers (500 ml). Each beaker filled with 500 ml tap water and covered with metal lid. Each batch of the prepared larvae were put into equal eleven (number of doses) groups, the group size was 20–30 larvae. The larvae were then submitted for X-ray. For *A. arabiensis,* adult males were put in groups (20-30 adults) for each dose, in a 500 ml beakers covered by mesh net that fixed in position with a rubber band.

The larvae and adults were then transferred to the laboratory of X-ray, National Cancer Institute (NCI). The samples were submitted to ten different scales of X-ray. The exposure time was for one flash shock for the treatment with doses of 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 and 5000 (rad) using X-ray radiation machine. These doses were selected according to some previous studies[16][17]. The irradiated larvae and adults were then transferred to the Blue Nile National Institute for Communicable Diseases (BNNICD) insectary under laboratory conditions set up at 28 °C and 75% RH. The fecundity (average number of eggs per female), fertility (percentage number of hatched larvae) and sterility (percentage number of un-hatched larvae) were recorded.

3.3 Statistical analysis

Appropriate data transformation was carried out when needed and then data were analyzed using simple descriptive statistics in addition simple regression and correlation statistics were executed.

4. Results

4.1 Fecundity

As were shown in Table (1) and Table (2), the average fecundity of 198 *A. arabiensis* females mated with irradiated males with X-rays at their adult stage was 110.3 eggs per female. In the control group it was 147.3 eggs per female. The fecundity ranged between 118.1 eggs per female in the 500 rays group to 111.3 eggs per female in the 5000 rays group. The average fecundity of the 170 females mated with irradiated male with X-rays at their larval stage was 93.2 eggs per female. In the control group it was 129.1 eggs per female. The fecundity ranged between 152.7 eggs per female in the 500 rays group to 75.1 eggs per female in the 5000 rays group.

4.2 Fertility

The average fertility of the *A. arabiensis* females mated to males irradiated at their adult stage was 24.41%. The fertility ranged between 68.28% in the 500 rays group to 1.42% in the 5000 rays group. In the control group the fertility was 97.65% (Table, 1). But the average fertility of females mated to males irradiated with X-rays at their larval stage was 58.83%. The fertility ranged between 82.01% in the 500 rays group to 9.83% in the 5000 rays group in the control group the fertility was 99.9% (Table, 2). It was clear that, x-irradiation affected the fertility of the eggs produced by the females mated with males treated at their adult stage more than that treated at their larval stage. It was also clear that, fertility is dose dependant for both cases (R^2 was 0.907).

4.3 Sterility

The sterility for the non-irradiated females of *A. arabiensis* mated to males irradiated with X-rays at their adult stage at 500 rays' level, there was 31.72% sterility and at 5000 rays level, it was 98.58% (the average was 75.59%). In the control group the sterility was 2.35% (Table, 1). Sterility due to doses in between 500 rays and 5000 rays ranged between 17.99% and 90.18%, respectively, for *A. arabiensis* females mated to irradiated males at their larval stage (the average was 41.17%). In the control group the sterility was 0.10%. It was clear that, sterility is dose dependant for both cases (R^2 was 0.909). Concerning the regression coefficient (X-coefficient), for each one unit increase in x-ray dose, a decrease of 0.016 and 0.018 units were occurred in fecundity and fertility, respectively, in the same time an increase by 0.019 unit was occurred in sterility of *A. arabiensis* females mated with males irradiated with x-ray at their adult stage. Also, for each one unit increase in x-ray dose, a decrease of 0.003 and 0.013 units were occurred in fecundity and fertility, respectively, in the same time an increase by 0.013 unit was occurred in sterility of *A. arabiensis* females mated with males irradiated with x-ray at their larval stage.

Table 1: Oviposition, Fertility and Sterility of the non-Irradiated females of *A. arabiensis* Mated to Males irradiated with X-rays at their adult stage

Dose (rays)	No. of females	Fecundity (NE/F)	Fertility (%No. of larvae)	%No. of (+) eggs	%No. of (-) eggs	Sterility (%)
Control	30	147.3	97.65	1.97	0.68	2.35
500	20	118.1	68.28	12.07	19.65	31.72
1000	15	144.4	42.29	18.05	39.67	57.72
1500	20	99.1	38.97	14.54	46.49	61.03
2000	20	93.9	32.64	20.13	47.23	67.36
2500	18	117.7	24.87	10.34	64.79	75.13
3000	19	92.8	12.36	11.12	76.52	87.64
3500	15	105.4	11.67	13.66	74.58	88.24
4000	15	99.9	8.01	12.74	79.25	91.99
4500	14	120.8	3.55	11.18	85.27	96.45
5000	12	111.3	1.42	7.26	91.32	98.58
Data analysis						
Mean		110.34	24.41			75.59
SE		15.71	6.72			6.72
Min.		92.8	1.42			31.72
Max.		144.4	68.28			98.58
Sum.		1103.4	244.06			755.86
R^2		0.06	0.907			0.907
Intercept		117.43	61.18			38.83
x-coefficient		-0.003	-0.013			0.013

(+) = Embryonated but unhatched eggs.
(-) = Unemberyonated eggs.
NE/F = No. of egg/females (Fecundity).

Table 2: Oviposition, Fertility and Sterility for the non-Irradiated females of *A. arabiensis* Mated with Males irradiated with X-rays at their larval stage

Doses (rays)	No. of females	No. of Eggs	NE/F (fecundity)	%No. of larvae (fertility)	%No. of (+) eggs	%No. of (-) eggs	% Sterility
Control	30	3873	129.1	99.90	0.10	0.0	0.10
500	19	2901	152.7	82.01	4.72	13.27	17.99
1000	19	2119	111.5	93.49	2.22	4.29	6.51
1500	17	1905	112.1	84.98	2.68	12.34	15.02
2000	16	1613	100.8	81.34	2.42	16.24	18.66
2500	14	1270	90.7	72.28	2.52	25.20	27.72
3000	14	1079	77.1	62.47	4.54	32.99	37.53
3500	13	916	70.5	45.52	4.15	50.33	54.48
4000	11	809	73.6	38.56	2.35	59.09	61.44
4500	9	611	67.9	17.84	0.0	82.16	82.16
5000	8	601	75.1	9.83	0.0	90.18	90.18
Mean			93.2	58.83			41.17
SE			8.45	9.31			9.31
Min.			67.9	9.83			6.51
Max.			152.7	93.49			90.18
Sum.			932	588			41.69
R^2			0.798	0.909			0.909
Intercept			136.55	109.78			-9.78
x-coefficient			-0.016	-0.018			0.019

(+) = Embryonated but unhatched eggs.
(-) = Unemberyonated eggs.
NE/F = No. of egg/females (Fecundity)±.

5. Discussion

From the literature, it is not clear exactly how the treated male can reduce the fecundity of the untreated female. The infecundity of female was attributed to the pairing and citus inability of the treated male [16]. In *A. arabiensis*, X-rays cause a significant decline in the percentage of larvae hatching at all doses [17].

In the mosquitoes, the comparable data on this point is lacking, though in *A. arabiensis*. A highly significant larval mortality was noticed at higher doses of gamma-irradiated but at 500 rays no significant differences could be found compared to the control [18]. Though the spade work of the effects of radiation on insects was done by many earlier researchers but Muller for the first time clearly identified and defined the term dominant lethal mutation who later described them as a result of chromosome break [19]. Subsequently, it was found by many studies (the review of which is beyond the scope of the present work) that dominant lethal mutation are caused by chromosome aberrations. This has been found also in many mosquito species cited above, including *Anopheles stephensi* [12]. However, many aspects of the effects of ionizing radiation on the biology of *A. stephensi*, the important malaria vector of the Middle East, Pakistan and India, remain to be explored.

6. Conclusions and Recommendations

The average fecundity of A. *arabiensis* females mated with irradiated males at their adult stage was 110.34 eggs per female. In the control group it was 47.3 eggs per female, whereas, the average fecundity of females mated with irradiated males at their larval stage were 93.2 eggs per female (in the control group it was 129 eggs per female). The fertility of female's *A. arabiensis* mated to irradiated males at their adult stage ranged between 1.42% and 68.28%, while it was 97.65% in the control group. The fertility of females mated to irradiated males at their larval stage ranged between 9.83% and 93.49%, while it was 99.9% in the control group. The sterility of *A. arabiensis* females mated to irradiated males at their adult stage ranged between 31.72% and 98.58%, while it was 2.35% in the control group. The sterility of females mated to irradiated males at their larval stage ranged between 6.51% and 90.18%, while it was 0.10% in the control group. Fertility and fecundity were dose dependant, while fecundity in the female mated with males treated at their adult stage showed very week correlation with doses. More studies should be conducted in order to well understand the best way of applying this method to control mosquitoes.

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