

# EFFECTS OF GAMMA RADIATION ON THE ACTIVITY OF THE MOSQUITO *CULEX PIFIENS* L.

## II. EFFECTS ON THE SEXUAL VITALITY OF THE MOSQUITO

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### ABSTRACT

The effects of 3 gamma doses on the sex vitality of the mosquito *Culex pipiens* L. were studied after irradiation of the larvae, pupae or newly emerged adults.

No significant effect on the sex attraction was observed when irradiation was done at the larval or adult stages with 40 Gray. However, there was a clear effect of radiation at all doses tested on the attraction of males to females when they were irradiated in the pupal stage. At 80 and 120 Gray, there was a significant decrease, when irradiation was carried out in the adult stage.

When adults were irradiated at the larval stage, there was a decrease in insemination ability of the males irradiated at 40 Gray when caged with normal or irradiated females. This effect was increased when normal males were caged with irradiated females. At all mating combinations the insemination ability of adult mosquitoes irradiated at the pupal stage was decreased with increasing the dose. When males were irradiated and confined with normal or irradiated females the insemination ability was less than when females were irradiated and confined with normal males, at all the doses tested. When newly emerged adults were irradiated at 40 Gray there was no effect on the male insemination ability. At 80 and 120 Gray, there was a slight effect on insemination ability of the male. As a whole, the effect of all doses on females was higher than on males.

When adults were irradiated at the larval stage, there was a clear decrease in insemination frequency of the male irradiated at 40 Gray when caged with normal or irradiated females. At all mating combinations the insemination frequency of

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adults irradiated at the pupal stage was decreased with increasing the dose. When the male was irradiated and confined with normal or irradiated females, the insemination frequency was less than when females were irradiated and confined with normal male at all the doses tested. When newly emerged adults were irradiated at 40 Gray, there was no effect on the male insemination frequency. However, at 80 and 120 Gray, there was a slight effect on insemination frequency of the male. The effect of irradiation at all doses tested on female was higher than on male.

## INTRODUCTION

In a previous work of this series of research papers, the effects of gamma irradiation on the orientation of the mosquito to habitat were investigated (Wakid *et al.*, in press). The present study was achieved to show the effects of the same gamma doses (40, 80 and 120 Gray) on the sexual vitality of the adult mosquito including sex attraction, ability to inseminate and insemination frequency.

## MATERIALS AND METHODS

The rearing technique of the mosquito and irradiation procedure were discussed in a previous paper (Wakid *et al.*, in press). The apparatus used in the experiments of that paper was also used in the sex attraction experiment of the present study. Radiation was applied to larvae (3rd larval instar), pupae (one-day old) or newly emerged adults. Females (irradiated or normal) were confined in the central cage, and males (irradiated or normal) were contained in the outer ones. All cages were left without darkening. Numbers of males leaving each of the outer cage i.e. attracted to the females, were counted. Three replicates were done.

### **Experiments of insemination ability**

In these experiments adults used were previously irradiated in their larval, pupal or adult stages as described before, at 0, 40, 80 or 120 Gray.

Equal numbers of previously isolated males and females of newly emerged adults were caged together and four mating combinations were made:

- I. Normal males × Normal females (control)
- II. Irradiated males × Irradiated females
- III. Irradiated males × Normal females
- IV. Normal males × Irradiated females.

For each combination the newly emerged adults were caged (in a rearing cage 25 × 25 × 25 cm.) and offered a cotton soaked with 10% sugar solution, then left for 3 days after which females were isolated and anaesthetized. They were dissected in a saline solution under a dissecting microscope to expose their sperma-thecae which were pressed by the cover-slide to investigate the presence or absence of sperms.

Mobile sperms were observed in inseminated females while no sperms were observed in the non-inseminated ones.

### Experiments of Insemination Frequency

The same mating combinations and procedure of the insemination ability experiment were followed here except that five females (normal or irradiated) were brought together with one male. The number of inseminated females by the male was recorded in each combination.

## RESULTS AND DISCUSSIONS

### 1. Sex Attraction

It was suspected that a response to sound was involved in mating (Maxim, 1901) but it was not until Roth (1948) investigated the mating behaviour of *Aedes aegypti* in detail that the significance of sound in mating was clearly demonstrated. The fact that a resting female might be surrounded by males, some even touching her, but that no male would attempt to copulate with her until she took to flight convinced Roth that odour was involved in mating behaviour. Sound was clearly implicated, however, since males were attracted to the source of sounds of certain frequencies and showed a characteristic mating response.

The present study aimed at the role of gamma radiation in the sex attraction. Here, trials were made to study the effect of irradiation on the attraction of males by females.

Figure (1) shows the effect of gamma radiation applied to the larval, pupal or adult stages on the attraction of males to females.

Irradiation of the third larval stage with 40 Gray resulted in males without any significant effect on their attraction to the normal males. Normal males were also attracted normally to the irradiated females.

When one-day-old pupae were irradiated, the irradiated males showed significant decrease in their attraction to normal females at all doses tested.

The effect was found to be slightly less when newly emerged adults were irradiated. There was no significant decrease at 40 Gray, while at higher doses there was a significant effect.

The data showed also that the effect was much more pronounced in the pupal irradiation than in the adult irradiation, this may be explained by the fact that adult germ cells are more resistant to radiation than those of the pupae.

Effects of gamma radiation on mosquitoes

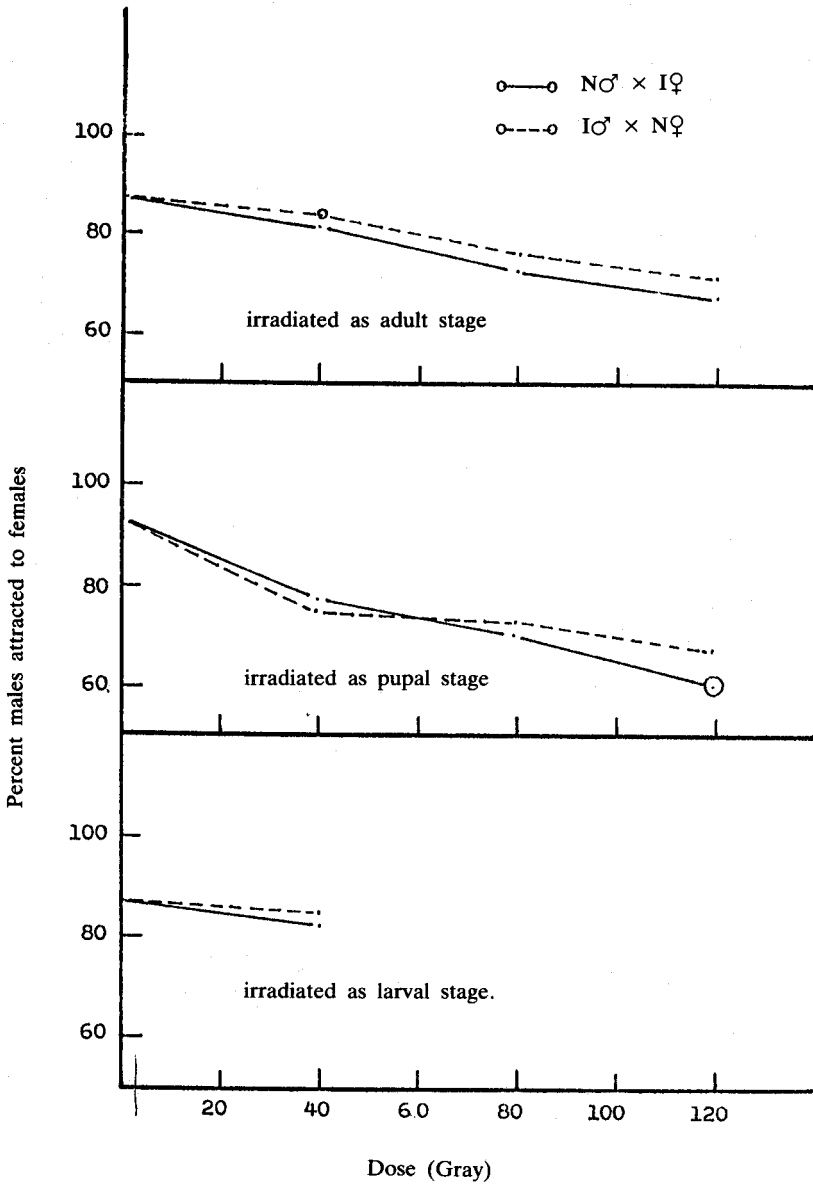


Fig. 1: Effect of gamma radiation applied in the larvae, pupae or adult stages on the attraction of males to females of *Culex pipiens*.

## 2. Ability to Inseminate

A primary requirement of the sterile insect technique for population suppression or eradication is that released sterilized insects must successfully mate with the wild population. To be successful the released insects and the wild ones must be compatible, mating propensity (speed) of the released must be high and the time of mating of released and wild insects must be synchronized. The technique followed was previously described before.

Tables (1-3) show the effects of 3 doses of gamma radiation on the ability of the male mosquito to inseminate or the female to be inseminated after irradiation at the larval, pupal or adult stages.

When the third instar larvae were irradiated with 40 Gray the produced adults showed a clear reduction in their insemination ability which was slightly increased (75.5%) when irradiated males were caged with irradiated females. When irradiated females were caged with normal males only 71.7% of them were inseminated. These results showed that both males and females were affected by gamma radiation in their ability to inseminate (males) or to be inseminated (females). This effect although being slight, however, shows that the radiation injury during the larval stage may appear in the adult stage (Table 1).

**Table 1**

Effect of gamma radiation applied to the third larval instar on the ability of insemination of 3-day-old adults *Culex pipiens* when equal numbers of males and females were caged and when one male was caged with 5 females (3 replicates).

Dose (Gray)	%Inseminated females in the different mating combinations $\pm$ S.D.			
	*N♂ × N♀	*I♂ × I♀	I♂ × N♀	N♂ × I♀
	When equal numbers of males and females were caged (insemination ability)			
40	86.6 $\pm$ 5.8	75.5 $\pm$ 13.2	73.3 $\pm$ 11.5	71.7 $\pm$ 20.2
80	No emergence			
120	No emergence			
	When one male was caged with 5 females (insemination frequency)			
40	73.3 $\pm$ 11.5	46.7 $\pm$ 11.5	40.0 $\pm$ 0.0	46.7 $\pm$ 11.5
80	No emergence			
120	No emergence			

\*N = Normal, I = Irradiated.

No data were obtained for 80 and 120 Gray, as adults were not produced after irradiating the larvae.

When one-day-old pupae were irradiated with 40 Gray, (Table 2) the produced

adult males showed a clear reduction in their ability to inseminate normal females (72.0%). This reduction was increased when the irradiated males were caged with irradiated females, being 64.4% in comparison with 90.4% in the control (normal males × normal females) and 90.2% when the normal males were caged with irradiated females. Increasing the dose to 80 Gray, a more pronounced reduction in the insemination ability of the adults was observed. However, the reduction was much lower in case of caging irradiated females with normal males. The latter case showed that radiation affects the insemination ability of the males more than that of the females, when irradiation was applied to the pupal stage. A drastic effect of gamma radiation on the insemination ability of both males and females was observed at the higher dose; 120 Gray. Only 40.0% of the irradiated females were inseminated by irradiated males, and 50.2% of the normal females were inseminated by irradiated males at 120 Gray, compared with 90.5% when normal males were caged with normal females. Again at the dose 120 Gray, the radiation affected the insemination ability at the pupal stage. This was clear because while only 50.2% of the normal females were inseminated by irradiated males, 67.1% of the irradiated females were inseminated by normal males.

Table 2

Effect of gamma radiation applied to the one-day-old pupae on the ability of insemination of 3-day-old adults *Culex pipiens* when equal numbers of males and females were caged and when one male was caged with five females (3 replicates.)

Dose (gray)	%Inseminated females in the different mating combinations ±S.D.			
	*N♂ × N♀	*I♂ × I♀	I♂ × N♀	N♂ × I♀
	<u>When equal numbers of males and females were caged (insemination ability)</u>			
40	90.4 ± 7.1	64.4 ± 13.9	72.0 ± 14.1	90.2 ± 14.7
80	88.7 ± 8.9	63.5 ± 13.8	52.2 ± 8.4	79.2 ± 15.5
120	90.5 ± 1.04	40.0 ± 12.9	50.2 ± 11.9	67.1 ± 19.5
	<u>When one male was caged with 5 females (insemination frequency)</u>			
40	73.3 ± 11.5	40.0 ± 0.0	40.0 ± 0.0	60.0 ± 20.0
80	73.3 ± 11.5	33.3 ± 11.5	33.3 ± 11.5	53.3 ± 11.5
120	73.3 ± 11.5	26.7 ± 11.5	26.7 ± 11.5	53.3 ± 11.5

\*N = Normal, I = Irradiated

Table (3) illustrates the radiation effects at three gamma doses, 40, 80 and 120 Gray applied to the newly emerged adults on the insemination ability of these adults when 3-day-old and caged in an equal numbers of both sexes.

The data obtained showed that at 40 Gray, there was no evidence of any effect on the male insemination ability. On the other hand, a slight effect, although

insignificant, was shown regarding male insemination ability. A slight effect was also observed at 80 Gray. This shows that at this dose of gamma radiation the females were more affected than males regarding their insemination ability, a result which is opposite to that obtained when irradiation was applied to the pupal stage. A quite similar effect was shown at the dose 120 Gray.

These results are similar to those obtained by many other authors working on different mosquito species. For example, Sonada (1972) reported that mating activity of the sterilized male *Culex pipiens molestus* was lower than that of normal males.

Many other authors reported the failure of the sterilized males to compete with the normal ones, e.g. Tantawy *et al.*, (1967) on *Anopheles pharoensis*; Abdel-Malek and Ahmed (1973), Patterson *et al.*, (1977) who found that irradiated males of *Culex pipiens quinquefasciatus* were 0.25 - 0.5 times as competitive as native males.

### 3. Insemination frequency:

This experiment was carried out in a trial to investigate the number of inseminations which a normal or irradiated male could perform when caged with enough numbers (five) of normal or irradiated females and also the number of normal or irradiated females that could accept insemination when caged with one normal or irradiated male. In other words, to study the effect of gamma radiation on the insemination frequency of the male.

From Table (1) it appears that gamma irradiation with the dose 40 Gray applied to the third larval instar gave adults with sharply reduced insemination frequency. This means that the dose of 40 Gray had a great effect in reducing the insemination frequency of the male. This also shows that gamma irradiation at 40 Gray affected the insemination acceptance of the females. The data also showed that one irradiated male could inseminate 46.7% of the irradiated females. As high doses of gamma radiation (80 and 120 Gray) affected the growth and survival of the immature stages, no adults were produced at these doses and consequently no data were taken in this study.

Irradiation of one-day-old pupae produced adults drastically affected in their insemination frequency. The data are presented in Table (2).

At 40 Gray only 40.0% of the normal females were inseminated by one irradiated male, compared to 73.3% of the normal females inseminated by one normal male. The effect was increased by the dose 80 Gray where only 33.3% of the normal females were inseminated by one irradiated male (73.3% in the control). This increase in effect at 80 Gray was also observed in the female acceptance to be inseminated. At the higher dose, 120 Gray, the effect on the male was increased while that on the female was equal to that at 80 Gray.

**Table 3**

Effect of gamma radiation applied to the newly emerged adults on the ability of insemination of 3-day-old adults *Culex pipiens* when equal numbers of males and females were caged and when one male was caged with 5 females (3 replicates).

Dose (gray)	%Inseminated females in the different mating combinations $\pm$ S.D.			
	*N $\sigma$ $\times$ N $\text{f}$	I $\sigma$ $\times$ I $\text{f}$	I $\sigma$ $\times$ N $\text{f}$	N $\sigma$ $\times$ I $\text{f}$
	<b>When equal numbers of males and females were caged (Insemination ability)</b>			
40	86.7 $\times$ 5.8	85.5 $\pm$ 2.1	87.8 $\pm$ 10.7	84.7 $\pm$ 2.4
80	90.0 $\pm$ 0.0	80.2 $\pm$ 5.4	86.9 $\pm$ 12.5	73.8 $\pm$ 2.1
120	86.7 $\pm$ 5.8	73.8 $\pm$ 5.6	86.7 $\pm$ 11.5	72.2 $\pm$ 6.9
	<b>When one male was caged with 5 females (insemination frequency)</b>			
40	80.0 $\pm$ 0.0	80.0 $\pm$ 0.0	80.0 $\pm$ 0.0	60.0 $\pm$ 0.0
80	73.3 $\pm$ 11.5	46.6 $\pm$ 11.5	60.0 $\pm$ 0.0	53.3 $\pm$ 11.5
120	73.3 $\pm$ 11.5	46.6 $\pm$ 11.5	53.3 $\pm$ 11.5	46.6 $\pm$ 11.5

\*N = Normal, I = Irradiated.

In the case of pupal irradiation the effect of gamma radiation on the female was less than that on the male.

Adult irradiation (Table 3) resulted in an effect which is less than that at pupal irradiation. At 40 Gray, it was found that while no effect was observed on the male insemination frequency, on the contrary the females were affected in their acceptance to be inseminated at 80 Gray, while 73.3% of the normal females were inseminated with one normal male, only 60% of the normal females were inseminated by one irradiated male. Irradiated male could also inseminate 46.6% of the irradiated females. The effect on females was also more than on males, as only 53.3% of the irradiated females were inseminated by one normal male. At the dose 120 Gray, the effect was more pronounced on both males and females. It is worthy to note that while males were more affected than females when pupae were irradiated, the opposite was true when irradiation was applied to the newly emerged adults.

The decreased mating frequency was not much reported on mosquitoes. However, Wakid (1965) working on *Anopheles pharoensis* stated that the laboratory male could mate with 4-5 females with an average of 4.5 females, while the wild male could mate with 5-6 females with an average of 5.5 females. This showed that wild male seemed to be more active sexually than the laboratory bred, although the difference was insignificant.



These results of the decreased sex attraction whether the male or the female was irradiated confirm and explain those obtained on the insemination ability where it was found that there was a drastic reduction in the percentages of females inseminated and in the reduced insemination frequency of males. Naturally as a result of the decreased sex attraction, the mating and insemination decrease.

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## تأثير أشعة جاما على نشاط بعوضة كيولكس بينيز

### ٢ - التأثير على النشاط الجنسي للبعوضة

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يشتمل هذا البحث على تأثير أشعة جاما بثلاث جرعات هي ٤٠ ، ٨٠ ، ١٢٠ جراي على النشاط الجنسي لبعوضة الكيولكس بينيز . ودلت النتائج المتحصل عليها على ما يأتي :

- لم يكن هناك تأثير ملحوظ على الانجذاب الجنسي عندما شععت الحشرات في الطور اليرقني بالجرعة ٤٠ جراي بينما كان هناك تأثير ملحوظ لجميع الجرعات المستعملة على انجذاب الذكور للاناث عندما شععت الحشرات في طور العذراء . أما عند الجرعة ٤٠ جراي فلم يكن هناك تأثير يذكر على انجذاب الذكور للاناث عند التشعيع في الطور البالغ ، بينما عند الجرعتين ٨٠ ، ١٢٠ جراي فكان هناك نقصاً في الانجذاب .
- عندما شععت الذكور في طور اليرقة بالجرعة ٤٠ جراي كان هناك نقصاً في قابليتها لنقل الحيوانات النوية إلى الإناث المشععة أو غير المشععة بينما زاد هذا النقص عندما شععت الإناث بنفس الجرعة وكانت الذكور غير مشععة . أما عند تشعيع العذارى فقد قلت قابلية النقل المنوي عند جميع المجموعات التزاوجية بزيادة الجرعة الاشعاعية . وعندما شععت الذكور وتراجدت مع إناث مشععة أو غير مشععة كانت قابلية النقل المنوي أقل منها عند تشعيع الاناث وتواجدت مع ذكور غير مشععة وذلك عند جميع الجرعات الاشعاعية المستعملة . وعند تشعيع الطور اليافع بالجرعة ٤٠ جراي لم يكن هناك تأثير ملحوظ على قابلية نقل الذكور المنوي بينما عند ٨٠ ، ١٢٠ جراي كان هناك تأثير بسيط على قابلية الذكر لنقل المنوي . وعلى العموم فإن تأثير الجرعات على الانثى كان أكثر منه على الذكور .

- عند تشعيع الحشرات في الطور اليرقي بالجرعة ٤٠ جراي أي كان هناك نقص في عدد مرات تزاوج الذكر المصحوب بنقل الحيوانات المنوية عندما يتواجد مع إناث عادية أو مشععة وفي جميع المجموعات التزاوجية كان عدد مرات التزاوج للحشرات المشععة في طور العذراء يقل بازدياد الجرعة الإشعاعية وعند تشعيع الذكر وتواجده مع إناث مشععة أو غير مشععة كان عدد مرات التزاوج أقل منه عند تشعيع الانثى وتواجدها مع ذكور غير مشععة في جميع الجرعات الإشعاعية المستعملة . أما عند تشعيع الحشرات في الطور اليافع بالجرعة ٤٠ جراي لم يكن هناك تأثير على عدد مرات التزاوج ، بينما عند الجرعتين ٨٠ ، ١٢٠ جراي كان هناك تأثير بسيط وعلى العموم فإن تأثير الإشعاع على الأنثى كان أكثر منه على الذكر عند جميع الجرعات المستعملة .