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Studies on the application of the sterile-male technique on the tick *Hyalomma excavatum*

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Summary: A sterile-male technique was studied on the tick *Hyalomma excavatum* and its applicability was discussed. The adult unfed male *Hyalomma excavatum* ticks were exposed to gamma irradiation at the dose of 15 Gray (Gy). The effects of irradiation on the male *Hyalomma excavatum* ticks were evaluated by the fertility performances of the untreated *Hyalomma excavatum* females which they were paired with. At the dose of 15 Gy, irradiated males were proved to be effective in competing with untreated males. Feeding times were prolonged, engorged weights and egg production were reduced, and egg viability and hatchability were significantly decreased. Since the results are expressive and promising, a sterile male release, in spite of obvious difficulties, could be considered feasible. However, further trials are needed to investigate if the sterility is transmitted from generation to generation without recovery of fertility.

Key words: Gamma irradiation, *Hyalomma*, sterile-male technique, tick.

Hyalomma excavatum kenesinde steril erkek tekniğinin uygulanması üzerinde araştırmalar

Özet: *Hyalomma excavatum* üzerinde steril erkek tekniğine uygun çalışmalar yapılarak tekniğin sahaya uygulanabilirliği araştırılmıştır. Gama radyasyonun *Hyalomma excavatum* üzerindeki etkisi, ışınlanmış erkek kenelerle çiftleşen ışınlanmamış dişi *Hyalomma excavatum* kenelerinin üreme performansları izlenerek incelenmiştir. 15 Gray (Gy) dozda ışınlanan erkek kenelerin ışınlanmamış kenelerle rekabet edebildiği görülmüştür. Kenelerin beslenme süreleri uzamış, düşme ağırlıkları ve yumurta verimleri azalmış, yumurtaların canlılığı ve yumurtalardan çıkan larva sayısı önemli oranda düşmüştür. Elde edilen sonuçlar steril erkek tekniğinin, uygulamadaki tüm güçlüklere rağmen kene kontrolü amacıyla kullanılabileceğini göstermektedir. Yine de, bu tekniğin sahaya uygulanmasından önce, gama ışınlamanın kenelerin üreme performansları üzerindeki etkisinin izleyen nesillere aktarılıp aktarılmadığı konusunda çalışmalar yapılması uygun olacaktır.

Anahtar sözcükler: Gama radyasyon, *Hyalomma*, kene, steril erkek tekniği

Introduction

Ticks are obligatory blood sucking Arachnids that feed on vertebrates. Ticks and tick-borne diseases affect animal and human health worldwide and are the cause of significant economic losses. Ticks act not only as vectors of a broad range of pathogens of domestic animals and humans but also cause damage directly due to their feeding behaviour (7).

The genus *Hyalomma* (Koch,1844) belongs to the largest family of ticks, Ixodidae. *Hyalomma* species serve as vectors and reservoirs for a variety of pathogens in humans and animals such as Q fever, Crimean Congo Haemorrhagic Fever, Babesiosis and Theileriosis (3,16). Sometimes ticks carry several pathogens simultaneously (19). *Hyalomma excavatum* is mainly found in the warmer climates of the Mediterranean, however it has been known to survive in areas with a marked winter (3). *Hyalomma excavatum*, the known vector of *Babesia sp.* and *Theileria annulata*, was also proved to be the reservoir of *Coxiella burnetii* (14, 20).

There are three major reasons for controlling ticks: Disease transmission, tick paralysis or toxicosis and tick-caused physical damage. Among the control methods, the indiscriminated application of acaricides creates undesirable effects on environment and human health (25). The increasing resistance of ticks to pesticides is another adverse effect (5,18). A well established, successfull approach to this problem should be the use of biological agents or methods (11,17).

The sterile-male technique is a method of biological control. In this technique sterility is generated by gamma irradiation (1). When sterile hybrid males are released to the area of problem, they compete with the wild males for the females. If a female mates with a sterile male then it will have less or no larvae, thus reducing the next generation's population. In theory, as females pass the

male sterility on to the next generations, repeated release of sterile males can eventually control or eliminate the population of insect in question. The estimated ratio of sterile to fertile male is 9:1 as the number of both types would be reduced by 50%. Even without further releases the population would decrease by 50% for the elimination of each generation, as long as there is no deterioration in sterility through the generations (6,12).

Srivastava and Sharma (21,22) reported the effects of gamma irradiation on different stages of *Hyalomma anatolicum* and found 20 Gray (Gy) dose as the sterilizing dose for males. Doses of 16 Gy or above were found to be effective on feeding and moulting of unfed nymphs of *Rhipicephalus appendiculatus* (26). Galun et al. (4) noted that males emerging from nymphs irradiated more than 20 Gy were not competitive due to lack of sperm. Males of *Rhipicephalus appendiculatus* were found to be more sensitive to the effects of gamma irradiation and at 40 Gy male ticks were all sterile but still competitive (15). Percentage egg weights, mean engorged weights, viability of eggs were also found to be reduced at the dose of 20 Gy (15,21). Oliver & Stanley (13) observed no hatch of larvae from crosses of irradiated *Ambylomma americanum* males and untreated females at 20 Gy or higher.

The results of a series of experiments for the application of sterile male technique in two other common species of *Hyalomma* were previously reported (8-10). Briefly, groups of unfed adult male *Hyalomma anatolicum* ticks were given single doses of gamma irradiation at range 0-50 Gy. Ticks were uneffected at radiation doses below 5 Gy but at this level and above feeding times were extended, engorged weights, egg viability and production were reduced (8). Males irradiated at 10 and 15 Gy were proved to be sterile but competitive. Above this level the competitiveness was low (8,9). Similar results were received in the unfed adult male *Hyalomma marginatum* ticks, and 15 Gy dose was stated to give more stable results than the dose of 10 Gy. So, for the further trials 15 Gy was used as a steril-male dose (10). This study is the continuation of these previous studies.

In this study, a model similar to a sterile male technique was designed and applied to unfed adult male *Hyalomma excavatum* ticks, and its practicality was discussed.

Materials and Methods

Ticks: The adult *Hyalomma excavatum* ticks were maintained from Aydın, Adnan Menderes University, Veterinary Faculty, Department of Parasitology. Unfed adult male *Hyalomma excavatum* ticks were exposed to 15 Gray (Gy) irradiation.

Experimental animals: For feeding the ticks, 4-6 months old conventional rabbits were used (approved by Gazi University Animal Experiments Local Ethics Committee, date: 20.04.2009 and No. B.30.2.GÜN.0. 05.06.00/68-6501). The ears of rabbits were cleaned and shaved three days before the placement of ticks and covered with cotton sacks. The ears were controlled everyday for the case of oedema. One day before the the application of ticks, rabbits were injected subcutaneously with 0.5cc dexamethazone for the inhibition of possible immune reaction.

Gamma Irradiation: Gamma irradiation was performed at the gamma irradiation facility of the Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Ankara. A ^{137}Caesium source with an effective dose rate of 0.532kGy/h was used for the irradiation. Batches of 10 unfed male ticks were placed inside the chamber of the gamma source and irradiated for the appropriate time to achieve the desired gamma irradiation doses. The exposure time for irradiation at the required dose was calculated according to the equation.

$$D = R \times T$$

where
D: radiation dose (Gy)
R: dose rate (Gy/h)
T: irradiation time (h)

Protocol: On the subsequent day following irradiation, irradiated groups of unfed male ticks were paired off with untreated unfed female *Hyalomma excavatum* ticks and placed on the ears of rabbits according to the protocol described in Table 1. Sterile male release conditions were taken into account while creating the first tick group. The groups 2 and 3 were used to control the effects of irradiation and considered together in the evaluation of the results. Two replicates of untreated male and female tick groups were used as controls (Group No. 4).

Table 1. Placing protocol of irradiated and unirradiated ticks on rabbit ears.
Tablo 1. Işınlanmış ve ışınlanmamış kenelerin tavşan kulaklarına yerleştirilme protokolü

Rabbit Groups	Right Ear	Left Ear
1	10F + 9IM + 1UM	10F + 9IM + 1UM
2	12F + 12IM	12F + 12IM
3	12F + 12IM	12F + 12IM
4	12F + 12UM	12F + 12UM

F: Female (untreated)
M: Male
I : Irradiated
U: Untreated

Evaluation: The effects of gamma irradiation on the tick groups were assessed by recording the behaviour (competition, attachment and survival rates) and feeding of the ticks on the host, and the subsequent reproductive performances of untreated female ticks mated with irradiated males. The engorged females dropped off the

host were collected daily and kept in individual bottles at 28ºC and 85% humidity. During the experiment following parameters were recorded: (a). Time to engorge, (b). Engorged weights of females, (c). Time to oviposition, (d). Time to egg hatch, (e). Number of eggs and (f). Number of larvae.

Results were calculated statistically by using SPSS package programme. For the distribution of parameters, Kolmogorov-Smirnov test was used. Statistical significance between the parameters showing normal distribution was tested by using Analysis of Variance (ANOVA). Kruskal-Wallis and Chi-Square tests were used for statistical significance of the other parameters.

Results

Irradiation of males at 15 Gy was appeared to be effective on the engorgement and oviposition of females (Table 2). Irradiation seemed to have an effect neither on the rate of attachment nor on the survival rate. The irradiated male ticks were competitive with the untreated males. In the groups 1 and 2-3, in which irradiated male ticks mated with unirradiated females, following major effects were observed:

- Comparatively fewer females engorged to normal level in matings.
- Engorgement weights were low and engorgement durations were prolonged.
- The number of female ticks having larvae were low and the statistical difference between these females in the group 1 and 2-3 and control group was significant ($p<0.01$).
- Number of eggs and number of hatch of larvae were significantly reduced.
- Hatchability of eggs was very low and limited number of larvae were weak, both slow in motion and pale in appearance (Fig 1, IIIa).
- Immature larvae either failed to hatch or stayed in the eggs (Fig 1, II-IVd,e).

Discussion

The unfed male *Hyalomma excavatum* ticks were exposed to gamma irradiation at the dose of 15 Gy for examining the practicality of sterile male technique. The results indicated that 15 Gy would be a suitable dose for the production of sterile *Hyalomma* ticks. This observation is close to that of Srivastava & Sharma (22) who suggested 20 Gy as a sterilizing dose for males of *Hyalomma anatolicum*. Galun et al. (4) recommended more than 20 Gy as the suitable dose for inducing sterility in males of *Ornithodoros tholozani* while Purnell

Table 2. The effects of irradiation on feeding and reproductive parameters of untreated adult female *Hyalomma excavatum* ticks mated with males treated at 15 Gy.
Tablo 2. 15 Gy dozda ışınlanmış erkek kenelerle çiftleşen ışınlanmamış dişi *Hyalomma excavatum* kenelerinin beslenme ve üreme parametreleri üzerine ışınlamanın etkileri.

Parameters	Groups	Number of irradiated and untreated males in the groups	x/n	Mean± St. deviation	Statistical significance
Time to engorgement (day)	1	1U + 9I	18/20	10.59 ± 0.76^{ab}	$p<0.05$
	2-3	12 I	27/48	11.29 ± 0.35^{b}	
	4 C	12U	22/24	9.45 ± 0.36^{c}	
Engorgement weight (g)	1	1U + 9I	18/20	0.53 ± 0.19^{a}	$p<0.001$
	2-3	12 I	27/48	0.34 ± 0.05^{b}	
	4 C	12U	22/24	0.80 ± 0.06^{c}	
Time to oviposition (day)	1	1U + 9I	18/20	7.09 ± 0.72	$p>0.05$
	2-3	12 I	27/48	5.92 ± 0.45	
	4 C	12U	21/24	6.50 ± 0.50	
Time to hatch of larvae (day)	1	1U + 9I	13/20	24.29 ± 0.75^{a}	$p<0.001$
	2-3	12 I	15/48	28.26 ± 0.18^{bc}	
	4 C	12U	21/24	28.24 ± 0.46^{c}	
Number of eggs	1	1U + 9I	18/20	5780.00 ± 905.37^{a}	$p<0.001$
	2-3	12 I	27/48	3551.48 ± 709.17^{b}	
	4 C	12U	21/24	8527.95 ± 807.40^{c}	
Number of larvae	1	1U + 9I	13/20	961.70 ± 321.26^{a}	$p<0.001$
	2-3	12 I	15/48	346.60 ± 124.38^{b}	
	4 C	12U	21/24	7104.04 ± 748.44^{c}	

x/n : Number of female ticks evaluated/total number of female ticks in the groups
C : Control group of untreated males and females
U : Untreated
I : Irradiated
^{abc} : Statistical significance is important between the different named groups in the same column
$p<0.001$: 95% confidence interval is statistically significant
$p>0.05$: 95% confidence interval is not statistically significant

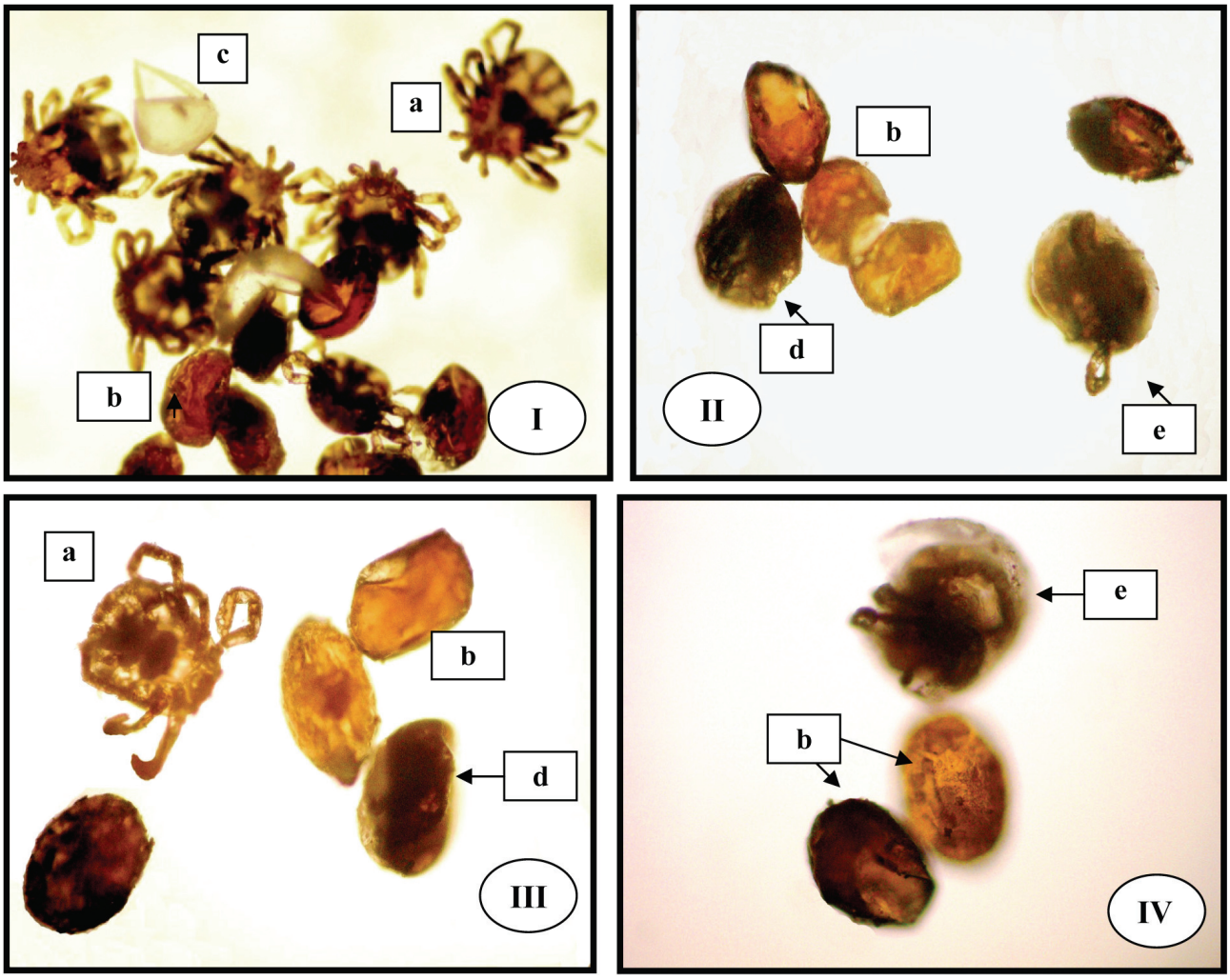

Fig 1. Microscopic scenes of eggs and larvae of ticks.
a. Larvae b. Eggs c. Eggshell d,e. Immature larvae
I: Control group. Hatchability rate was high, immature larva was not seen;
II-IV: Groups of crosses of gamma irradiated (15 Gy) males and untreated females of *Hyalomma excavatum*. Hatchability rate was low, limited numbers of larvae were weak and pale in appearance (III-a). Immature larvae either stayed in the eggs (II and III-d) or failed to hatch (II and IV-e).
Şekil 1. Kenelerin yumurta ve larvalarının mikroskopik görüntüleri.
a. Larva b. Yumurta c. Yumurta kabuğu d,e. Gelişmemiş larva
I: Kontrol Grubu. Yumurtadan larva çıkma oranı yüksek oldu, gelişmemiş larva görülmedi;
II-IV: Gama ışınlamaya tabi tutulan (15 Gy) *Hyalomma excavatum* erkekleri ile ışınlanmamış dişilerin çiftleştirildiği gruplar. Yumurtadan larva çıkma oranı çok düşük, az sayıdaki larva görünüş olarak soluk renkli ve güçsüzdü (III-a). Gelişimini tamamlayamayan larva ya yumurta içinde kalmış (II ve III-d) ya da yumurtadan çıkmayı başaramamıştı (II ve IV-e).

et al. (15) reported 40 Gy of gamma irradiation as the dose for sufficient reproductive sterility for adult males of *Rhipicephalus appendiculatus*. Bakri et al. (1) gave the radiosterilization dose as 32 Gy for the family Ixodidae. Drummond et al (2) who studied the effects of gamma irradiation on various stages of *Ambylomma americanum*, found that the reproductive performances of females mated with the males irradiated at the dose of 10 Gy were varied at the point of development. This result is in agreement with the result of Karaer et al. (10).

At 15 Gy dose, the irradiated males were competitive with normal males. The preliminary findings (8,9) had shown a decrease in competition at 20 Gy or higher. However, Galun et al. (4) suggested that *Ornithodoros tholozani* males, irradiated at 20 Gy or higher doses, were effective in competitiveness. Our observations showed that, the doses of 20 Gy or above had negative effects on competitiveness, and the attachment and survival rates.

The most marked effects of this study were shown on the feeding and fertility performances of normal females mated with males irradiated at 15 Gy. The engorgement duration prolonged, engorged weights, number of eggs and larvae were reduced. These findings are similar to those of Purnell et al. (15) who found that engorged weights, egg production rate, viability of eggs

and resultant egg hatches were all reduced at 20 Gy dose. Oliver et al. (13) observed no hatch of larvae from eggs of unirradiated females of *Ambylomma americanum* mated with males treated at 20 Gy. At the doses of 16 Gy or above feeding and subsequent development of unfed nymphs of *Rhipicephalus appendiculatus* were found to be impaired (26). Similarly, fewer unirradiated females of *Hyalomma excavatum* ticks mated with males treated at 15 Gy engorged to normal levels, and subsequently limited numbers of larvae were hatched.

The extention of feeding times for engorgement may also be due to the male factor, a substance produced by the male gonad of ticks and transferred to the female during mating and responsible for the transition to rapid engorgement (23). Weiss & Kaufman (24) who studied on the male gonad of *Ambylomma hebraeum*, suggested that the male factor and the engorgement factor were the same substance. It is possible that engorgement factor proteins may not be synthesized during feeding as a result of irradiation, and females, mated with irradiated males, are not able to to complete their engorgement because of lack of these proteins.

It appears that sterile male technique could be feasible at the dose of 15 Gy, in spite of obvious difficulties in practice. However, further trials are needed to investigate if the sterility transmitted from generation to generation without recovery of fertility. As the feeding and fertility systems of ticks are complex, it will be useful to work on the relationship between the male factor and ionizing radiation.

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