CHAPTER 7

EFFECTS OF GAMMA RADIATION ON THE LARVAL AND POST-LARVAL STAGES OF *ANTHRENUS VORAX*

Studies on the radiosusceptibility of a number of stored-grain pests have indicated the possibility of using ionizing radiations for their control by breaking the developmental cycle or by inducing sterility. In many insects, doses in the range of 15 to 20 kilo rad are known to cause reproductive sterility and death of the stadium within a few weeks (Nicholas and Wiant, 1959; Cornwell and Morris, 1959; Horne and Brownell, 1962; Nair and Rahalkar, 1963). *Anthrenus vorax* being a common pest of wool, leather, horn and many other stored materials of animal origin, it was thought desirable to obtain data on its susceptibility to different doses of gamma radiation.

The effects of ionizing radiations on the different life stages of insects have been adequately reviewed by Grosch (1963). Recently Nair (1962) presented evidence to show that in house fly pupa, susceptibility to gamma radiation was inversely proportional to the degree of differentiation of the tissues. Donnelly (in Nair, 1962) also found a marked decrease in susceptibility with advancing age in blow fly pupa. It should be of interest, therefore, to study this phenomenon in *Anthrenus vorax* which belongs to a different Order of insects. In the present investigation, the larval and post-larval stages of *Anthrenus vorax* were exposed to different doses of gamma radiation and

their effects on moulting and pupation of the larva, as well as emergence, fecundity and fertility of the adult, were studied. Observations were also made on the influence of radiation on ovarian development.

MATERIAL AND METHODS

The insects were obtained from the laboratory culture maintained at 32 ± 1°C on a diet of dried and crushed pigeon breast muscle supplanted with 5% Brewer's yeast.

Irradiation was carried out in a Cobalt - 60 source lodged at the Atomic Energy Establishment, Trombay, Bombay. A dose-rate of 1.5×10^5 rad/hour was employed.

The larvae used were of the last instar which were identified by their size. This, however, included a certain number of the previous instar, an estimate of which could be obtained from the control group. In the pupal stage, the males and females were irradiated and observed separately, but the data are pooled together, since there was no significant difference in the effects between the two.

Dissections of the ovaries were made after staining them *in situ* with Toluidine blue. The dissected out ovaries were fixed in Bouin's fluid for half an hour, washed and photographed immediately.

RESULTS AND DISCUSSION

Irradiation of the larva

The results obtained on the response of the larval stage to different doses of gamma radiation are presented in Table I.

TABLE I

EFFECTS OF GAMMA RADIATION ON THE LARVAL STAGE OF ANTHRENUS VORAX

Dose k rad	Pupation (%)	% of larvae which reached the prepupal stage	Total mortality (%)	Mortality on the 42nd day of irradiation (%)	Attempted moults (%)	% of successful moults based on the attempted moults
0 (175)*	98.29	98.29	2.29	-	45.14	100.00
4 (75)	5.33	44.00	94.67	89.3	61.33	71.73
5 (175)	1.71	45.14	98.86	86.0	38.86	73.53
10 (174)	0.57	15.52	99.43	84.8	40.82	35.21
15 (100)	1.00	24.00	100.00	58.0	24.00	33.33
20 (75)	5.33	5.33	94.67	54.7	40.00	23.33

* Figures in parentheses denote the total number of insects used in each dose group. The data consist of the results of two sets of experiments.

It may be seen that pupation was more or less completely inhibited at all dose levels employed. However, in all dose groups, a few larvae underwent successful pupation either directly after irradiation or after moulting repeatedly with concomitant reduction in size and subsequent regrowth. Those pupated directly died in the early pupal period, whereas the others emerged as normal adults and produced normal viable eggs. Of the larvae which underwent repeated moulting and reduction in size, however, many died as small wasted larvae.

Mortality occurred within about two months after irradiation. The percentage mortality at the end of 42 days were 89.3, 86.0, 84.8, 58.0 and 54.7 for those treated with 4, 5, 10, 15 and 20 k rad respectively (Table I), which shows that at lower doses, death occurred within a shorter length of time than at higher doses.

The irradiated larvae, as indicated earlier, consisted of the last instar as well as the last but one which in the normal course of development would undergo a further moult. Thus it was possible to study the effect of radiation on the process of moulting. Since the larvae were assigned to the different dose groups at random, it could be expected that all the groups would contain a proportionate number of the two instars. The percentage of larvae, in each dose group, which attempted to moult and the percentage of which were completed successfully, are shown in Table I. The percentage of successful moults decreased with increasing doses of radiation. Thus at doses of 4 and 5 k rad, 71.7%

and 73.5% respectively of the attempted moults were completed successfully, whereas, at doses of 10, 15 and 20 k rad the corresponding percentages were 35.2, 33.3 and 23.3 respectively. In other words, at higher doses comparatively more of the larvae died in the process of moulting - either at a stage preparatory to moult (which could be reckoned by a blackening and elongation of the body) or at a partly moulted stage. The available data do not indicate any definite influence of radiation on the process of initiation of moult.

At lower doses (4 & 5 k rad), comparatively more of the larvae reached the prepupal stage before they succumbed to the radiation injury (44.0% with 4 and 45.1% with 5 k rad), whereas, at higher doses (10, 15 & 20 k rad) the majority of them died in the larval stage itself, only 15.5% in 10 k rad treated, 24.0% in 15 k rad treated and 5.3% in 20 k rad treated larvae reaching the prepupal stage.

The present study shows that Anthrenus vorax, in comparison with another dermestid beetle, Trogoderma granarium, is more susceptible to gamma radiation in the larval stage. In the last instar larvae of Anthrenus, 95% inhibition of pupation was obtained with a dose of 4 k rad (which was the lowest administered) whereas, for the corresponding stage of Trogoderma, a dose level of 14 k rad was required for complete pupal inhibition (Nair and Rahalkar, 1963).

It is interesting to note that a few larvae in most of the dose groups were capable of undergoing pupation after

repeated moults, reduction in size and regrowth. However, it may be pointed out that only 11 out of the 700 larvae exposed to radiation had shown this behaviour. This phenomenon is all the more significant considering the fact that the adults thus produced were quite normal. It is not understood what factors are responsible for conferring such resistance on some of the larvae. Repeated moults were also observed in the irradiated larvae of a related beetle, Deremestes vulpinus (Nair, personal communication).

The finding that at lower doses the majority of the larvae died only after reaching the prepupal stage or the moult phase (depending on whether they came from the last instar or the previous one), is in conformity with the general observation that in the immature stages of insects, the lethal effects of radiation injury is manifested only during the next developmental crisis (Grosch, 1963). However, the doses 10, 15 and 20 k rad had caused a greater derangement of the normal biochemical processes so that the majority of the last stage larvae were incapable of entering the prepupal stage, and eventually succumbed to the radiation injury after a prolonged larval existence. This phenomenon of arrested growth, which is a well known effect of radiation, was responsible for the longer survival period of the larvae exposed to higher doses.

Irradiation of the prepupa

When the early prepupae were exposed to 5 k rad, 100% mortality was obtained, 81.1% dying as prepupa, 15.1% at the

pre-moulting stage and 4.8% after moult(no. of insects observed - 53). Late prepupae were exposed to 3 and 5 k rad. With 3 k rad, 100% of them (no. of insects observed - 32) pupated, of which 37.5% emerged as normal adults and 62.5% as deformed ones. Among the latter, some showed incomplete apposition of the elytra, some complete absence of scales, while some others possessed only a few scattered scales on the elytra and the body. Of those exposed to 5 k rad (no. of insects observed - 77), 94.8% pupated, 3.9% died as prepupa and 1.3% died after moulting. Of those pupated, 82.2% died on or before the 7th day of pupation, in 73.3% of which partial migration of the wing rudiments towards the dorsal side had occurred. No scales were formed on the elytra. The remaining 17.8% of the pupae died after the 7th day pupal stage, in which the dorsal apposition of the elytra was incomplete, but the scales were formed with the normal colour pattern.

It could be seen that as regards the larval-pupal moult, the extent of developmental progression at the time of irradiation had an important influence. The fact that the larval-pupal moult was unhampered by irradiation of the late prepupa in contra-distinction to those irradiated as early prepupa, indicates that there is a critical period between the early and late prepupa after which irradiation is ineffective in preventing pupation. It seems likely that the small number of moults among the early as well as late prepupa was due to incorporation of the earlier stages in the sample rather than a stimulatory effect of radiation on moulting. Due to the small number of prepupae available at a

particular time it was not possible to keep non-irradiated controls.

Among the morphological abberrations caused by irradiation of the prepupa, one point deserves special mention. The formation of adult scales was inhibited only when the insects were irradiated at the prepupal stage resulting in the emergence of adults either entirely or partially devoid of scales. Irradiated after this stage, the emerging adults had always possessed scales with the normal distribution pattern (see below). It is clear from these observations that though the scales make their appearance only after the 7th day of pupation, the prepupal stage is a critical period during which the development of the scales is determined.

Irradiation of the pupa

Table II presents the data obtained on the susceptibility of the various pupal stages to different doses of radiation. It is evident that susceptibility to a particular dose decreased with advance in the age of the pupa. Thus a dose of 5 k rad, for which complete data are available for all successive days of the pupal period, caused 100% mortality on the 1st day of pupation, but the percentage mortality decreased progressively on successive days till the 7th day, after which no mortality was observed. It can also be seen that at least during the initial stages of pupal life, mortality increased with higher doses for a particular stage of development. The most susceptible period was found to be the first 3 days of pupal life, beyond which even doses as high as 10 k rad did not have appreciable

TABLE II

EFFECTS OF GAMMA RADIATION ON THE PUPAL STAGE OF ANTHRENUS VORAX

Stage of development at the time of irradiation	Percentage mortality after a given dose of				
	2 k rad	3 k rad	5 k rad	8 k rad	10 k rad
1st day pupa	0.0 (41)*	-	100.0 (49)	-	-
2nd day pupa	0.0 (47)	41.2 (17)	100.0 (15)	-	-
3rd day pupa	0.0 (47)	-	86.2 (29)	-	-
4th day pupa	-	-	15.4 (26)	-	0.0 (48)
5th day pupa	-	-	5.8 (52)	-	0.0 (34)
6th & 7th day pupa	-	0.0 (39)	5.4 (57)	2.6 (39)	-
Pre-emergent adult	-	0.0 (90)	0.0 (236)	0.0 (90)	-

* Figures in parentheses indicate the total number of insects in each group which were irradiated as two or more batches.

effect on adult emergence. It may be noted that on exposure to 3 k rad, 41.2% of the 2nd day pupa died (and the rest were abnormal) as against the 100% survival (though 62.5% of the adults were deformed) of the late prepupa exposed to the same dose.

Marked transitions in radiosusceptibility with advance in age were also observed in the pupae of the house fly, Musca domestica (Nair, 1962) and the blow fly, Lucilia sericata (Donnelly, in Nair, 1962). This was attributed to the rapid progress of tissue differentiation taking place in the early pupal period, since studies on rat embryos (Wilson, 1954) have shown that susceptibility to gamma radiation is inversely proportional to the degree of differentiation of tissues (Nair, 1962). The present study provides further proof of the dependence of radiosensitivity on the progress of tissue differentiation. When we consider a particular morphological event such as the larval-pupal moult, the formation of scales, the formation of hind wings or the attainment of the adult form, there is always a particular stage beyond which irradiation has little effect on its successful completion.

Since the most susceptible period of the pupal life was the first three days, it may be concluded that the process of histogenesis is more or less completed during this period.

Exposure of the early pupae to sublethal doses of radiation caused certain malformations in the emerging adults. 1st day pupa exposed to 2 k rad produced 69% of normal adults and 31% of malformed ones, in which the scale formation was normal

but the hind wings were sac-like filled with some fluid (probably haemolymph). The under-surface of the elytra also showed some blisters. Saccular hind wings were also observed in many of the two k rad treated and all of the 3 k rad treated 2nd day pupae. In 2 k rad treated 3rd day pupa, only 10% of the emerged adults showed saccular hind wings. None of the pupae irradiated after the 3rd day, showed malformed hind wings. The appearance of blisters on the elytra and wings were also reported in Tenebrio molitor after exposure of the early pupae to X -radiation (Brockway, 1956). Imperfect apposition of the elytra was another common effect of irradiation; the percentage which showed such deformation depended on the dose level as well as on the stage of the pupa. Often the emerging adult was unable to free itself of the pupal exuvia which remained attached to the tip of the abdomen. Such an effect was also observed in Tenebrio after X -irradiation (Brockway, 1956).

The longevity of the adults from those irradiated at the 6th or 7th day pupal stage or the pre-emergent adult stage did not differ considerably from that of the normal adult, the average longevity in days being 19.9, 18.5 and 21.7 for the 6th and 7th day pupa treated with 3, 5 and 8 k rad respectively, and 21.2, 19.3 and 2 4.3 for the pre-emergent adult treated with 3, 5 and 8 k rad respectively. These averages are not strictly correct since an error of 2 to 3 days was allowed in registering the longevity of each individual. The average life span of the normal adult was 18.1 days. Adults produced from those irradiated at an earlier pupal stage showed a slightly shorter life span, but no attempt was made for a quantitative assessment of the difference.

Effect of radiation on fecundity and fertility

The effects of exposure of the pre-emergent adult and the 6th & 7th day pupa to different doses of radiation on the fecundity and fertility of the emerging adults, are presented in Tables III and IV. A normal mating pair produced an average of 52.7 eggs with an average viability of 98.7%. In contrast to this, a mating pair which received 3 k rad in the pre-emergent adult stage gave only 23 eggs of which only 1.075% were viable. On increasing the dose to 5 or 8 k rad, the fecundity was further reduced to 13 eggs per female and the viability fell to nil. When the irradiated female was crossed with normal male, the fecundity was 14.25, 14.60 and 3.0 for 3, 5 and 8 k rad treated females, respectively. The percentage viability in this case, showed a definite increase to 22.8 in 3 k rad treated female which fell to 1.37 in 5 k rad treated and nil in 8 k rad treated ones. In the cross between normal female and irradiated male, the number of eggs laid was significantly higher than when the female was irradiated, almost comparable to the control. The percentage of viability was, however, not different from that of the group in which both the parents were irradiated. Two points speak for themselves in the data presented here - one, that the irradiation of the female brings about a significant reduction in fecundity and the other, that, though the induced sterility of the male as well as the female contributes to the nonviability of the eggs, the male is more sensitive than the female as regards fertility.

It can be seen from Table IV that the fecundity of a

TABLE III

EFFECT OF EXPOSURE OF THE PRE-EMERGENT ADULT TO DIFFERENT DOSES OF GAMMA RADIATION ON THE FECUNDITY AND FERTILITY

Dose	I. female x I. male		I. female x N. male		N. female x I. male	
	Average no. of eggs per female	Viability (%)	Average no. of eggs per female	Viability (%)	Average no. of eggs per female	Viability (%)
3 k rad	23.25 ± 3.90	1.08	14.25 ± 1.09	22.80	48.25 ± 7.22	1.04
5 k rad	13.00 ± 2.51	0.0	14.60 ± 3.32	1.37	47.67 ± 3.30	0.0
8 k rad	13.60 ± 4.84	0.0	3.0 ± 3.33	0.0	40.50 ± 1.50	0.0

Data obtained from an average of 4 replicate observations of each mating combination.

' ± ' = Standard deviation. I = Irradiated N = Normal

TABLE IV

EFFECT OF EXPOSURE OF THE 6th & 7th DAY PUPA TO DIFFERENT DOSES OF GAMMA RADIATION ON THE FECUNDITY AND FERTILITY

Dose	I. female x I. male		I. female x N. male		N. female x I. male	
	Average no. of eggs per female	Viability (%)	Average no. of eggs per female	Viability (%)	Average no. of eggs per female	Viability (%)
3 k rad	20.25 ± 7.46	7.41	30.00 ± 6.16	24.16	46.75 ± 1.92	16.58
5 k rad	1.60 ± 3.20	0.0	0.0	0.0	39.00*	16.00
8 k rad	0.0	0.0	0.0	0.0	36.67 ± 11.32	8.18

Data obtained from an average of 4 replicate observations of each mating combination.

' ± ' = Standard deviation. ' I ' = Irradiated. ' N ' = Normal.

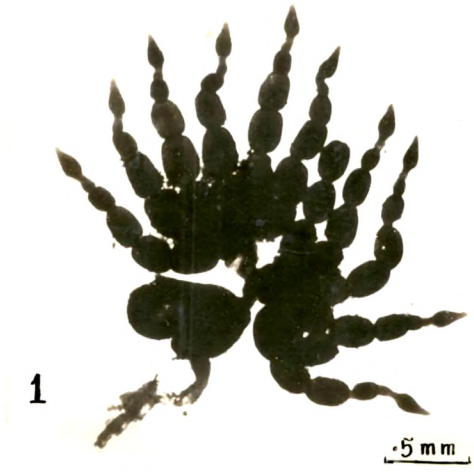
*Consisted of only one observation.

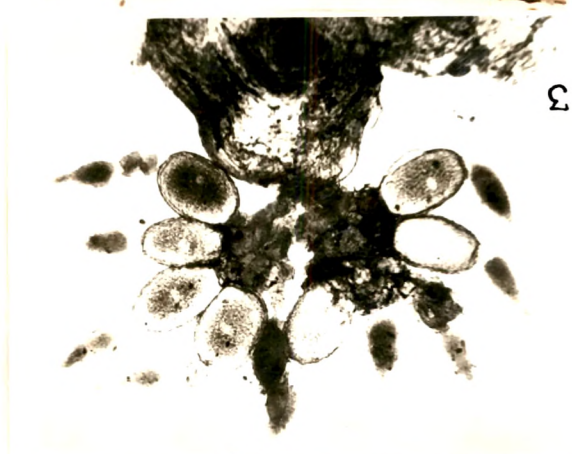
mating pair which received 3 k rad in the 6th or 7th day pupal stage did not differ significantly from that of the previous group (Table III), but the percentage viability showed an increase. With 5 & 8 k rad, there was a notable difference in the fecundity between the two groups, these doses being more detrimental to the fecundity of those treated at the 6th or 7th day pupa. The cross between irradiated female and normal male again shows a greater percentage of viability in the 3 k rad treated group. The complete absence of viability in the 5 & 8 k rad treated groups is mainly a reflection of the infecundity of the female. The cross between normal female and irradiated male, as in the previous group (Table III), shows a higher number of eggs per female, almost equivalent to the control. The percentage of viability in this case shows a marked increase from that of the previous group at all doses. The obvious conclusions are that, at this stage, the females are more sensitive to 5 & 8 k rad with respect to fecundity and that the male is less sensitive to all the doses with regard to fertility than at the pre-emergent adult stage.

It is clear from the above data that irradiation of the female at all dose levels employed, had an inhibitory effect on egg production. It is now well established that the developing ovarian tissues of insects are quite vulnerable to radiation (Grosch, 1963; Lachance and Bruns, 1963; Ross and Cochran, 1963). That the reduced number of eggs in the present case is due to inhibition of ovarian development, was ascertained by dissections

of the ovary in certain dose groups. All dissections were carried out on the first day of emergence of the adult. In this insect, egg production is autogenenous; on the 1st day of emergence a normal ovary consists of 6 ovarioles, each containing at least 3 well differentiated egg chambers (Fig. 1). When the insects exposed to 3 or 5 k rad at the pre-emergent adult stage (10th day of pupation) were dissected on the first day of emergence, it was seen that the ovarioles consisted of only one well developed egg chamber (Figs. 2 & 3). In normal female such a developmental stage of the ovary is reached in the pre-emergent adult stage itself, and evidently, the formation of subsequent egg chambers was inhibited by irradiation. The ovaries were much smaller, without any egg chamber, when the insects received the radiation (3 k rad) in the 6th or 7th day pupal stage (Fig. 4). The ovaries of the female which received 10 k rad at the 5th day pupal stage were found to be at a still earlier stage of development (Fig. 5).

The general effect of radiation on the ovaries, therefore, is an arrest of growth at the particular stage of development reached at the time of irradiation. However, on exposure to 3 k rad, the ovaries seemed to be capable of sustaining the development of nearly two batches of eggs, after an initial period of inhibition, whereas, on exposure to 5 or 8 k rad, the ovaries appeared to have been irreparably damaged, only those eggs which had already passed some critical developmental stage at the time of exposure being laid subsequently. The greater inhibition of





egends on

EXPLANATION OF THE PHOTOGRAPHS

Figures 1 - 5 show the state of development of the ovary on the day of emergence of the adult. The ovaries were stained in situ with Toluidine blue, dissected out and fixed for half hour in Bouin's fluid. They were photographed immediately after washing in water. The scale given in Fig.4 also applies to figures 2, 3 and 5.

1. Non-irradiated control
2. Exposed to 3 k rad at the pre-emergent adult stage
3. Exposed to 5 k rad at the pre-emergent adult stage (pressed under a cover slip)
4. Exposed to 3 k rad at the 6th or 7th day pupal stage
5. Exposed to 10 k rad at the 5th day pupal stage

fecundity when the insects were irradiated at the 6th or 7th day pupa is due to a greater susceptibility of the ovarian tissue at the earlier stages of development.

Regarding fertility, it may be mentioned that dominant lethal mutations must have been induced in both sperms and ova, but the sperms appear to be more sensitive in this respect. The greater sterility of the males irradiated at the pre-emergent adult stage compared to those irradiated at the 6th or 7th day pupal stage may be due to the fact that the active process of spermatogenesis takes place during the pre-emergent adult period.