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Radiosensitivity in larvae of tasar silkworm, Antherea proylei (Lepidoptera)

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ABSTRACT

The radiosensitivity in larval instars of tasar silkworm, Antheraea proylei (Lepidoptera) was studied by analyzing larval growth, frequency of larval mortalities, moulting and larval durations. The growth of larvae was inversely proportional to dose of gamma ray treated. The larval lethalities expressed in LD_{50} values were found to be 60 Gy, 115 Gy, 160 Gy, 210 Gy and 265 Gy in Ist, IInd, IIIrd, IVth and Vth instars respectively. As dose increases the larval durations were also increased in tolerable doses

INTRODUCTION

The importance of ionizing radiations as mutagenic agents has been fully appreciated during the recent years. According to latest scientific research, irradiation has immense potential and lots of applications. Hirobe (1974) stated that in silkworms, growth and other quantitative characters are changed by gamma irradiation depending upon dose rate, total dosage, developmental stage, temperature, moisture and other environmental conditions. Shamitha and Purushotham (2008) irradiated the diapauses cocoons of A. mylitta with 5Gy, 7.5Gy and 10Gy and observed significant reduction in pupal duration and increases in duration of moth stage along with morphological changes in moths. In the present study, an attempt is made to study the radiosensitivity of the different larval stages of Antheraea proylei through gamma ray irradiation. The present study on the radiosensitivity in larval instars of Antheraea proylei will allow to understanding the radiosensitivity at different developmental stages of the silkworms which will give a basis for further studie

MATERIAL AND METHOD

The hybrid tasar silkworm, Anthraea proylei Jolly (Lepidoptera, Saturnidae; n=49) collected from local government farms and reared in our laboratory constituted the experimental material. The larvae of different instars were treated with different increasing doses of gamma radiation (Dose rate = 0.81 Gy/sec) and then transferred to cut twigs of food plants, Quercus serrata rested in water bottles and covered with polyethylene bags under a thatch to protect from flies, birds and rats.

Doses given:

12 hr old Ist instar larvae after hatching---0, 50, 75, 100, 150, 200 and 250 Gy

12 hr old IInd instar larvae after first moult---0, 100, 125 and 150 Gy

12 hr old IIIrd instar larvae after second moult---0, 150, 175 and 200 Gy

12 hr old IVth instar larvae after third moult---0, 150, 175, 200, 225 and 250 Gy

12 hr old Vth instar larvae after fourth moult---0, 200, 225, 250, 275 and 300 Gy

OBSERVATION

Treated Ist to Vth instar larvae were observed for lethality twice a day. The number of death larvae in each replicate after treatment was noted. Number of moultings for respective instars and cocoons formed were also noted.

Evaluation of LD_{50} dose for larvae of A. proylei:

The percent mortality for each sample of exposed larvae was calculated and mortality curves were drawn against the dose for calculation of LD_{50} dose.

Lethal dose (LD_{50}):

The concept of 50% lethal dose as an endpoint for scoring radiation death has been borrowed from the field of pharmacology. The 50% lethal dose or LD_{50} is defined as the dose of any agent of material that causes a mortality of 50% in the experimental group, within a specified period of time (Hall, 1978).

Radiation sensitivity of the whole organism varies greatly according to the species, the strain and even the individual. In mammals, it is usually expressed in terms of $LD_{50/30}$, which represents the radiation dose required to kill 50% of animals within 30 days. However, the criterion can hardly be applied to the silkworm, which undergoes complete metamorphosis in a short period. Therefore, LD_{50} is used in the insect in a modified form: LD_{50} for hatching, LD_{50} from hatching to pupation or emergence, LD_{50} during the pupal stage from pupation to emergence etc. (Tazima, 1978). In the present study, the endpoint was taken as maturation of larvae just before spinning.

RESULTS

Effect of different doses of gamma radiation on growth of larvae:

For establishing the relationship between radiation dose and growth of larvae, 12 hr old larvae of A. proylei were treated with 0 Gy, 50 Gy, 75 Gy, 100 Gy, 150 Gy, 200 Gy and 250 Gy. Three replicates of 50 larvae in every replicate for every dose treatment were used. Up to 9 days after treatment, there were no differences in growth. After 9 days, the difference in growth and development could be noticed between the control and treated samples. The treated samples gradually started to show slow growth and development. The difference which was minute at first became prominent day by day, which was higher in greater dose treatments. Not only slow growth, but the stage is also retarded in treatments where the degree is directly proportional to dose. At the end of one month, only 0 Gy, 50 Gy and 100 Gy treated samples existed (Fig. I), while all others died gradually. The harvested cocoons show decrease in size as dose increases (Fig. II).

Radio resistance of instars in terms of LD_{50}:

Depending on the number of larvae survived up to the stage just before spinning, mortality rates were calculated for each dose in each instar and mortality curves were drawn against the doses (Fig. III, IV, V, VI and VII). Thus the LD_{50} values in Ist, IInd, IIIrd, IVth and Vth instars were found to be 60 Gy, 115 Gy, 160 Gy, 210 Gy and 265 Gy respectively. The curves resembled an elongated S shape in all instars.

Radiation effects on moulting and larval durations:

As a result of increasing doses of gamma radiation, the successive moultings were affected thereby affecting larval durations. More frequency of the larvae died in the moulting process. The process itself took more time if it happens to be completed. As a result, the larval durations demarcated by successive moultings were elongated in treated samples. The elongation of larval durations was such that for every treated sample, first there was an increase in the durations, and then as dose increases, the durations decreased (Figs. VIII, IX, X, XI, and XII). This might be because of enhanced death due to higher doses. The total larval durations demarcated from radiation exposure up to start of spinning are also changed due to radiation. In treated samples, the total larval durations were at first elongated as dose increases, but at high doses, the larvae cannot survive up to start of spinning (Figs. XIII, XIV, XV, and XVI).

Radiation sickness:

Radiation sickness is applied to the multiple physiological symptoms of the larvae. It includes lower food intake, dull movement, less and sticky excreta and mucous like secretion sometimes produced the excreta in chains. It also includes an oozing of haemolymph through the mouth (vomiting of greenish liquid).

Lethality after maturation of larvae:

Lethality after maturation of larvae was studied in IVth and Vth instar treatments. The lethalities after maturation of larvae were noted in the samples and were calculated in percentage of controlled value and the mean values were evaluated. The prepupal lethalities were 18%, 20%, 16%, 16% and 11% in 150 Gy, 175 Gy, 200 Gy, 225 Gy and 250 Gy treatments respectively in IVth instar treatments (Fig. XVII). The naked pupal lethalities were only 4%, 8%, 2%, 4% and 8% respectively in the doses mentioned. Most of the individuals which were capable of forming cocoons died in the pupal stage.

In Vth instar treatments, prepupal lethalities (Fig. XVIII) were 0%, 4%, 8%, 10% and 13% respectively in 200 Gy, 225 Gy, 250 Gy, 275 Gy and 300 Gy treatments. Naked pupal lethalities were 2%, 2%, 0%, 6% and 10% respectively. The frequencies became higher because of enhancement of death due to radiation injury at higher doses.

DISCUSSION

The present study aims at evaluating the effects of gamma radiation on the developmental stages of the economic hybrid species of tasar silkworm, Antheraea proylei and finally to draw the degree of radioresistance for commercial utilization. Radiosensitivity of an organism is usually the range of doses causing the animal to die with haemopoietic syndrome phenomena (Yarmonenko, 1988). The degree of radiosensitivity varies greatly within a single species (individual radiosensitivity), while for a definite individual, it depends on the age and sex. Moreover, wide variation in radiosensitivity in different cells and tissues is observed in the same organism in which sensitive tissues coexist with radioresistant ones (Yarmonenko, 1988). Survival or mortality curves are used for the quantitative studying of the radiosensitivity of an organism.

The LD_{50} values are important data for the comparison of radioresistance of the animals. In the present study, the LD_{50} values gradually increasing from 60 Gy in Ist instar to 115 Gy in IInd instar, 160 Gy in IIIrd instar, 210 Gy in IVth instar and 265 Gy in Vth instar at 12 hr after hatching or moult which shows that the radioresistance increases gradually as the metamorphological stage advances. In insects, the radioresistance of the species have been reported to be different at different metamorphological stages (Tazima, 1978). The LD_{50} values of silkworms are comparatively higher than those of other animals. The $LD_{50/30}$ values for sheep, rabbit, rat and desert mouse were found to be 1.55 Gy, 8.4 Gy, 9 Gy and 15.20 Gy respectively (Hall, 1978). The molecular rationale for this radioresistance in insects is believed to involve very efficient DNA repair processes (Koval, 1980) which allow them to maintain their genetic integrity. It is generally acknowledged that dose rate has a significant effect on the LD_{50} in mammals (Chee et. al., 1979). The LD_{50} for B. mori eggs, a few hr after oviposition, was 800R(=8 Gy); the eggs became more resistant with the progress of embryonic development reaching a plateau at day 7 when LD_{50} was as high as 6500R, both in non-dormant and dormant eggs (Akita et. al., 1965). They investigated the variation in radiosensitivity of eggs in relation to dormancy. When eggs were kept at 25 degree C throughout the egg stage, radiosensitivity gradually decreased from the plateau level of LD_{50}=6500R to a trough of LD_{50}= 2000R until the dormancy was broken 100 days after oviposition. In the present investigation, the temperature was maintained at 25±1 degree C throughout the experiment at 8 hr light treatment. The exact nature of the radio resistance in insects is unknown, but it has been attributed largely to the lack of, or minimal amount of, cell division in adult insects (O'Brien and Wolfe, 1964; and Ducoff, 1972). This explanation is based on the law of Bergonie and Tribondeau (1959) which states that the sensitivity of cells to radiation is directly proportional to their reproductive activity and inversely proportional to their degree of differentiation. However, on the contrary, recent studies on the TN-368 lepidopteran insect cell line have suggested that the cells of insects have an intrinsic radioresistance. The mitotically active cells of this line are approximately 50-100 times more radioresistant than mammalian cells (Koval, 1983). In another view, the radioresistance of lepidopteran cells has been attributed to be a consequence of their possession of holokinetic chromosomes (Bauer, 1967; Murakami and Imai, 1974; La Chance, 1974; and Suomalainen, 1953). Holokinetic implies that kinetochores or centromeres are spread out or diffused along the chromosome (White, 1973). The haemolymoh of silkworms contains cystathionine (Kondo, 1962) and lanthionine (Inokuchi, 1972), the synthesis of which increase gradually to the gradual increase of larval development. Both of these amino acids contain thiol (-SH) group which is generally found radioresistant. Thus the gradual increase of radioresistance with the larval developmental stages may be due to the increase in these amino acid syntheses and also due to the minimal cell division towards later developmental stages.

The growth and development have been observed to be retarded more when younger metamorphological stage is exposed to radiation. Acid phosphatase is known to provide phosphate to the tissues having high energy requirements especially during development, growth and maturation (Hurkadli et. al., 1985). So, the retardation in growth of the irradiated larvae might be due to some kind of damage in the acid phosphatase activity. The growth and development of the larvae are again found to be decreased with the increase in radiation dose. However, an increase in the specific activity of acid phosphatase was observed (Gadhia and Shah, 1981) in pigeon spleen following irradiation. Again an increase was observed in the specific activities of lysosomal hydrolases and Beta glucuronidase in rat spleen (Snyder and Eklund, 1978). In our study, it is found that the younger the stage irradiated and the greater the dose given, the greater is in the growth retardation. The effects of ionizing radiation on growth, lethality and reproduction of many species of plants have been previously reported (Fraley, 1971; Fraley and Whicker, 1973; Platt, 1963; and Woodwell, 1962). The reason behind restoration of growth of the larvae as a result of developmental age with the same dose might be due to the increase in radioresistance in older developmental groups.

According to the law of Bergonie and Tribondeau (1959), the sensitivity of cells to radiation is directly proportional to their reproductive activity and inversely proportional to their degree of differentiation. It implies that the sensitivity of cells to radiation will increase with the increase of reproductive activity and will decrease with the increase of differentiation. However, the results of the present study show that with the advancement of developmental stage, the reproductive activity i.e. gonadal development increases, and the animals of older developmental stage i.e. with more degree of differentiation become more radioresistant.

In the present study, increase of dose produced greater larval lethalities. The evaluation of radiosensitivity of Ist to Vth instar larvae exhibits dose dependent survivality. It has been already stated that radiosensitivity decreases gradually with the progress of larval stages towards maturity. Not only survivality, the other quantitative and qualitative characters decline significantly with increasing doses of radiations in each developmental stages. Similar results have been observed in other insect species (Menon, 1978; Srinivasan and Kesavan, 1979). Increased mortality with gradual increase of dose was also reported (Wharton and Wharton, 1959) in Periplaneta americana (L).

In the post irradiation development, most larvae died in the moulting period. It is noteworthy that higher doses of radiation prolong the larval periods due to delay in moulting process, but very high doses again shorten the periods as a result of hastened death due to radiation injury. Moulting is a hormonally controlled phenomenon. The hormone, ecdysone is produced during moulting in the versonian gland which is located behind the head and this intricately timed neuromuscular and neuroseculary mechanism controls the moulting process. Partial or whole body irradiation studies of larvae suggested that radiation might affect this hormonal regulation (Sivasubramanium et. al., 1974; Horikawa and Sugahara, 1960).

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FIGURES

Figure I

Figure II

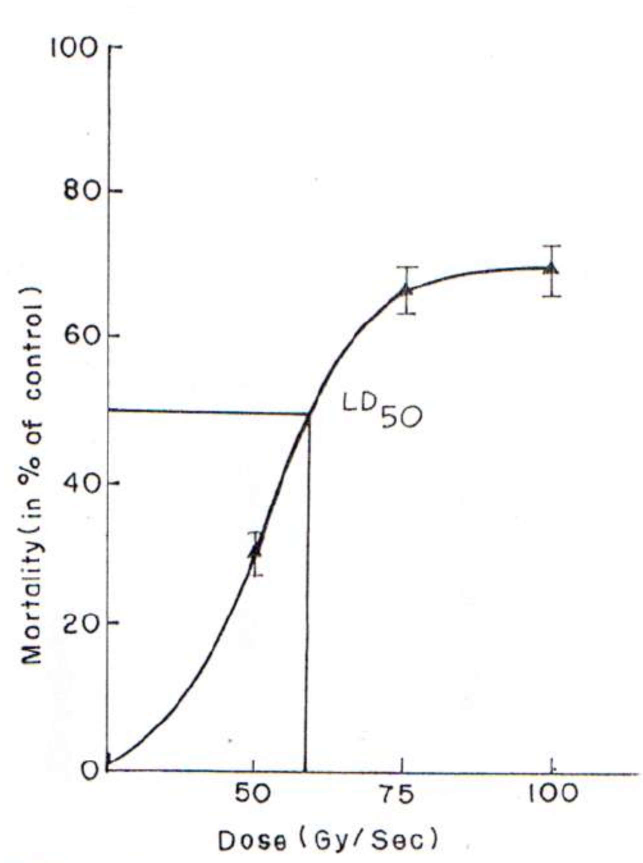
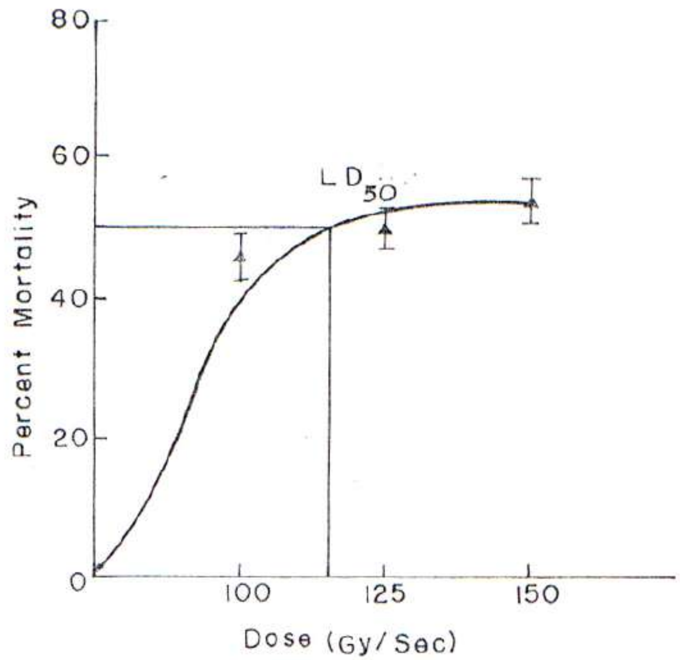

100
80
60
40
20
0
Mortality (in % of control)
LD50
50
75
100
Dose (Gy/Sec)

Figure III



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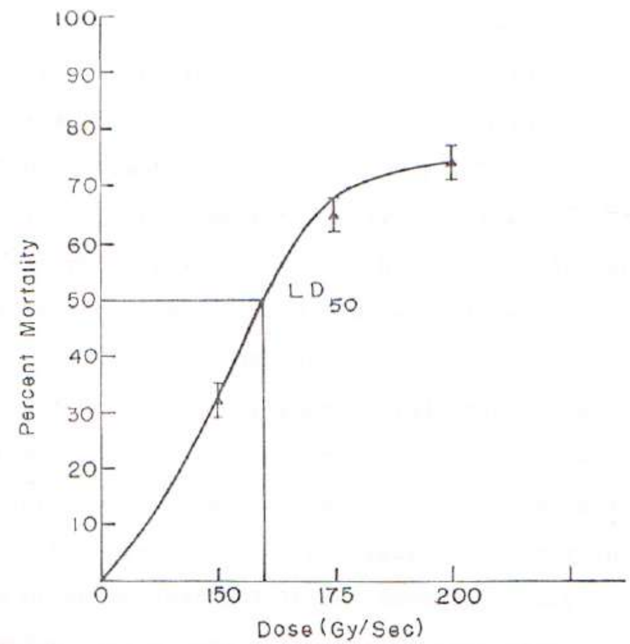

100
90
80
70
60
50
40
30
20
10
Percent Mortality
LD 50
0
150
175
200
Dose (Gy/Sec)

Figure V

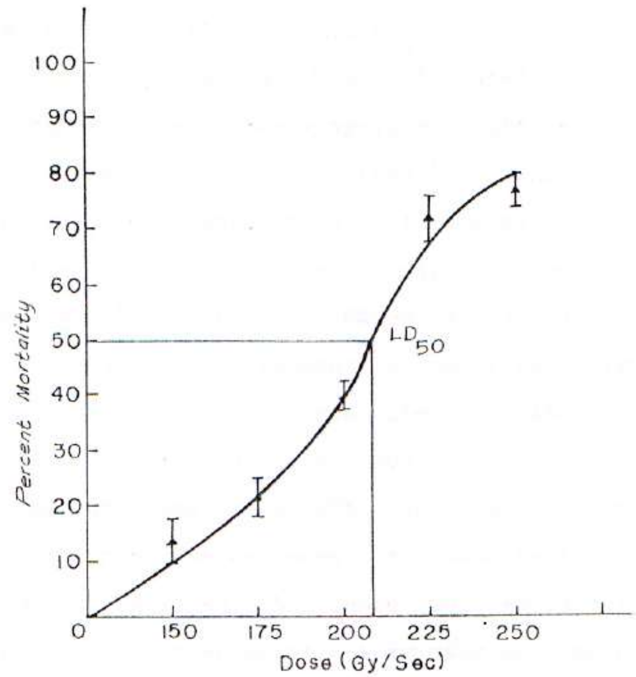

100
90
80
70
60
50
40
30
20
10
0
Percent Mortality
LD50
150
175
200
225
250
Dose (Gy/Sec)

Figure VI

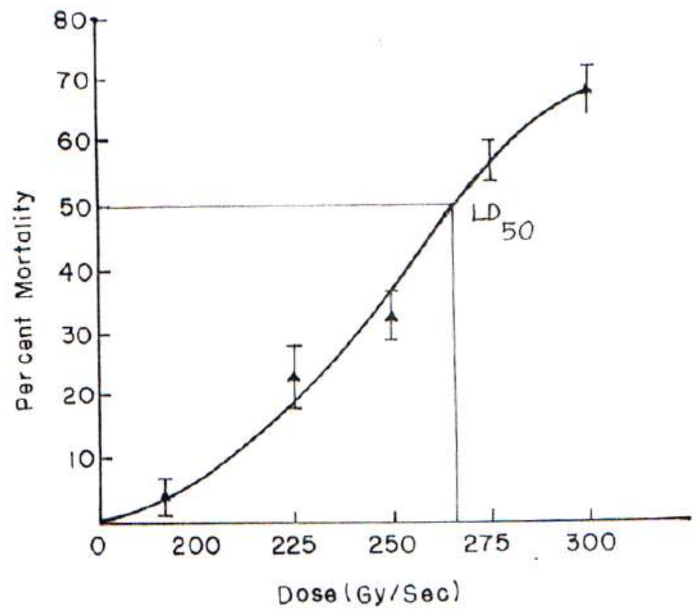
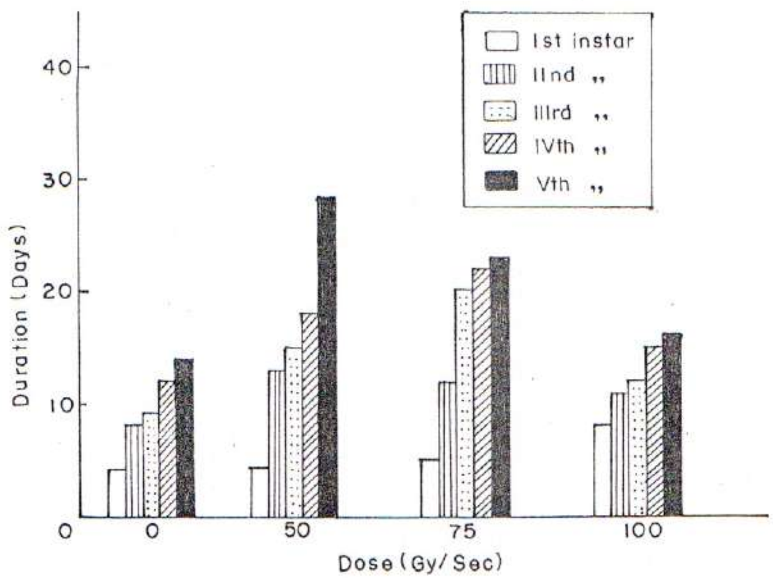
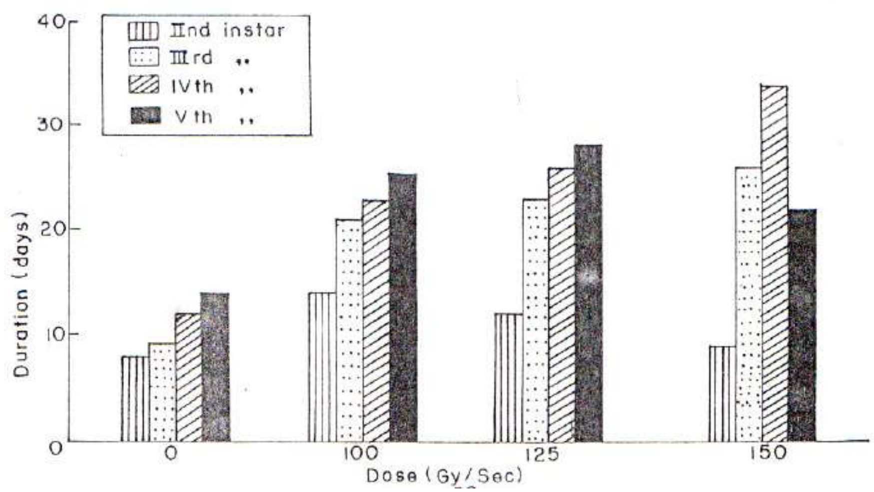

80
70
60
50
40
30
20
10
Per cent Mortality
LD50
0
200
225
250
275
300
Dose (Gy/Sec)

Figure VII



Ist instar
IInd ,,
IIIrd ,,
IVth ,,
Vth ,,
Duration (Days)
40
30
20
10
0
0
50
75
100
Dose (Gy/Sec)

Figure VIII



IInd instar
IIIrd ,,
IVth ,,
Vth ,,
Duration (days)
40
30
20
10
0
0
100
125
150
Dose (Gy/Sec)

Figure IX

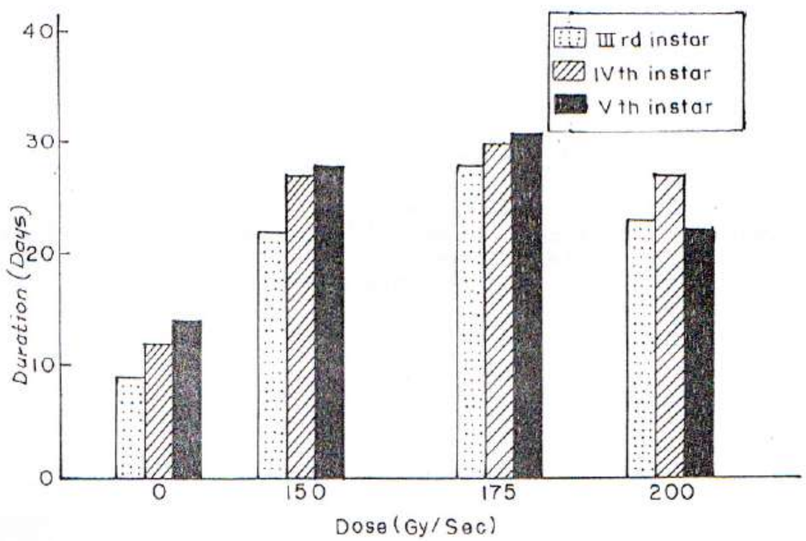


Figure X

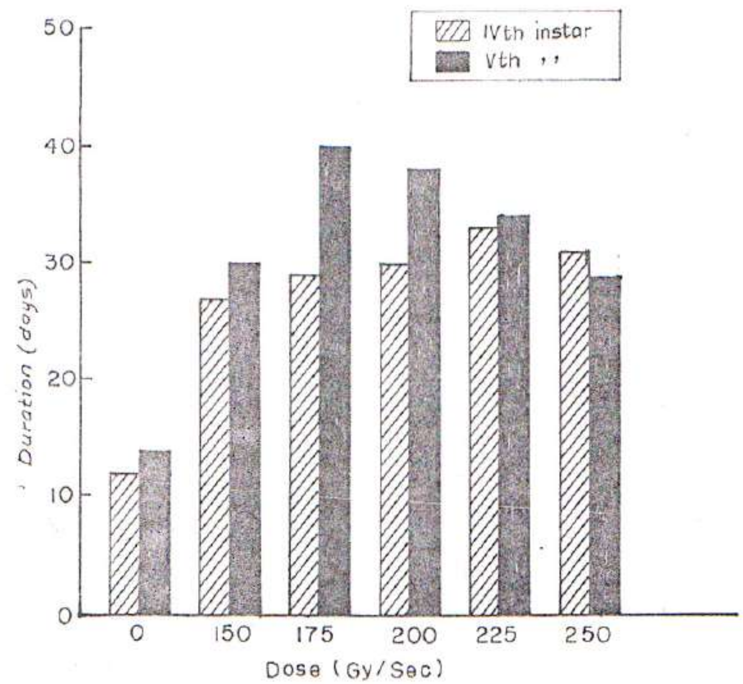

IVth instar
Vth ,,
50
40
30
20
10
0
Duration (days)
0
150
175
200
225
250
Dose (Gy/Sec)

Figure XI

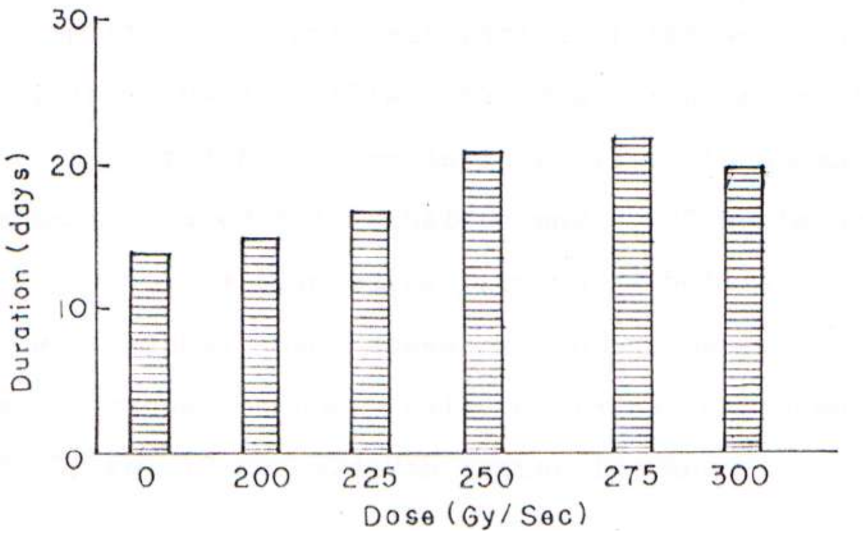
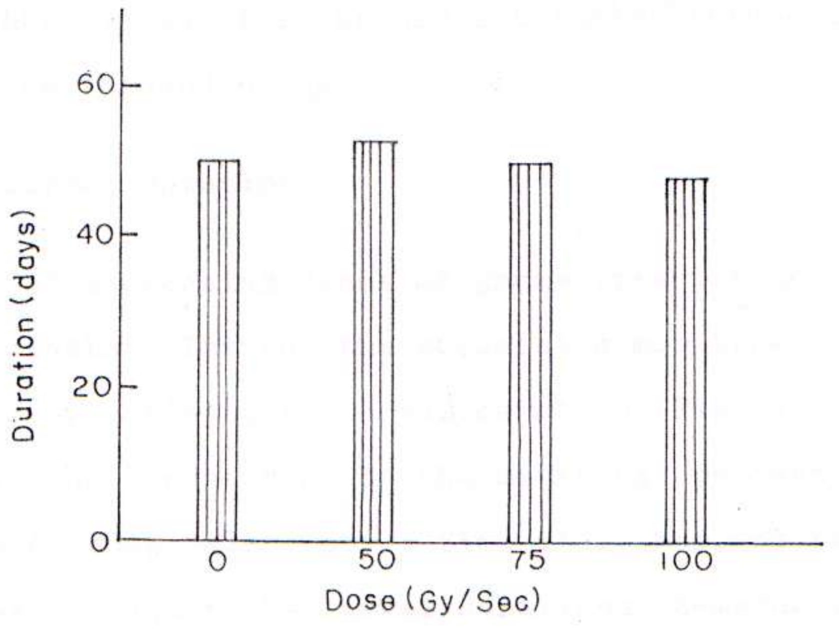
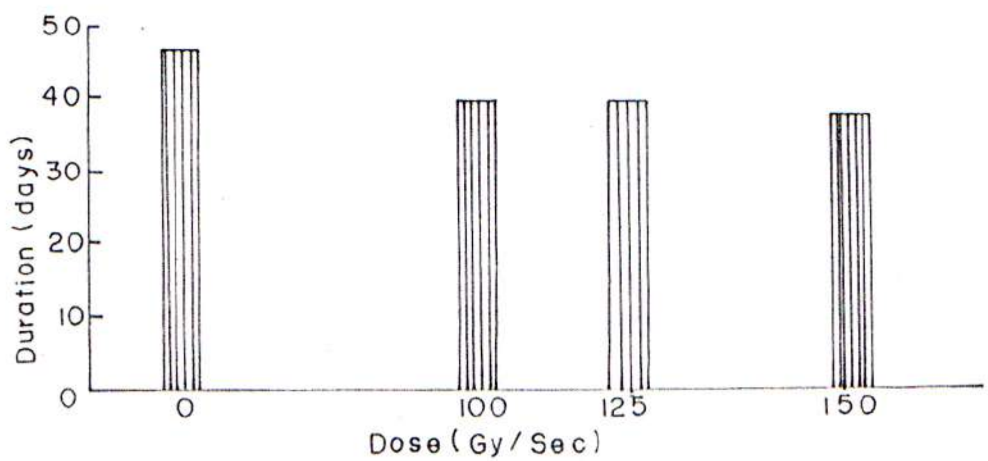


Figure XII



Duration (days)
60
40
20
0
0
50
75
100
Dose (Gy/Sec)

Figure XIII



Duration (days)
50
40
30
20
10
0
0
100
125
150
Dose (Gy/Sec)

Figure XIV

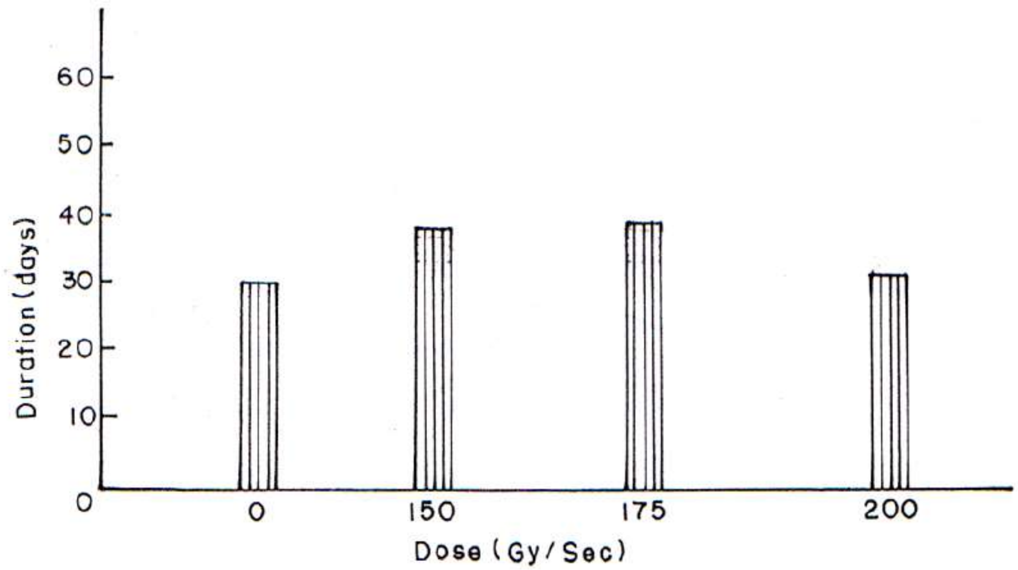


Figure XV

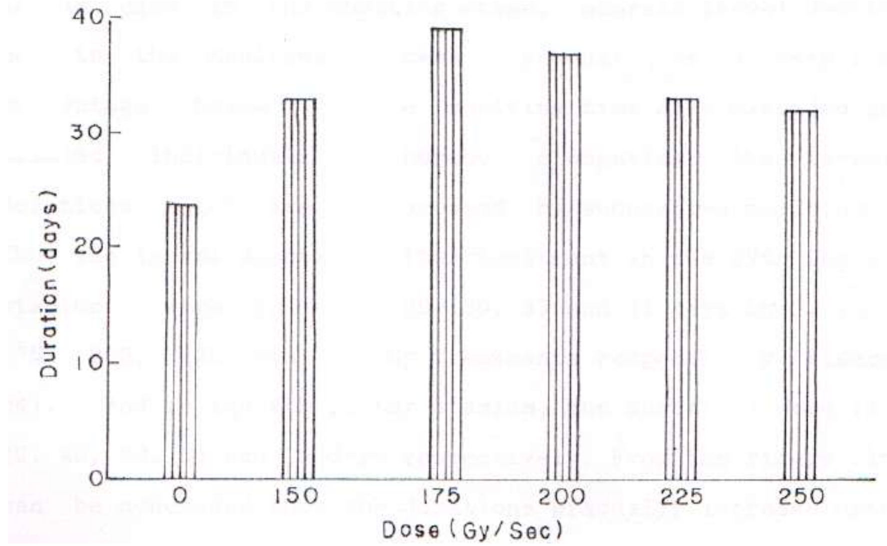


Figure XVI

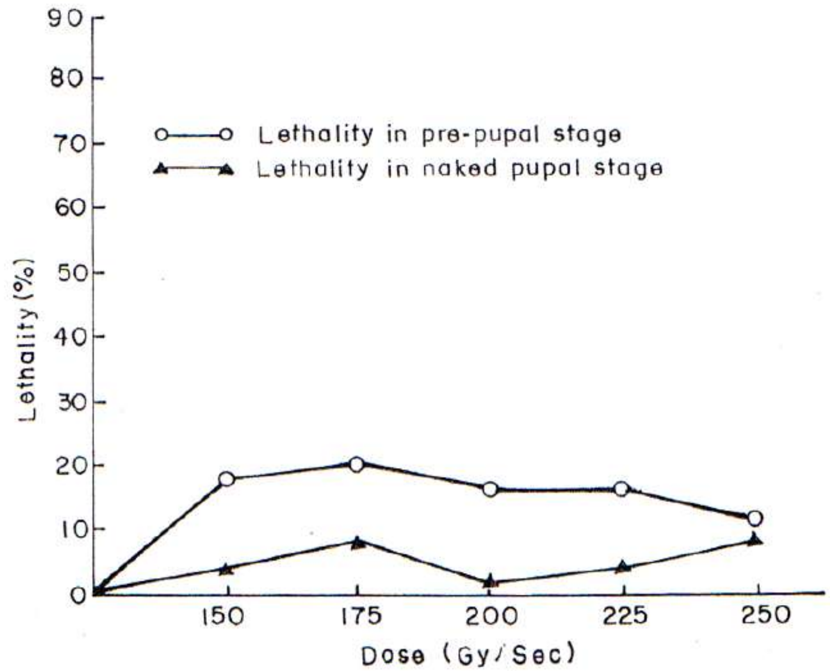

Lethality in pre-pupal stage
Lethality in naked pupal stage
Lethality (%)
90
80
70
60
50
40
30
20
10
0
150
175
200
225
250
Dose (Gy/Sec)

Figure XVII

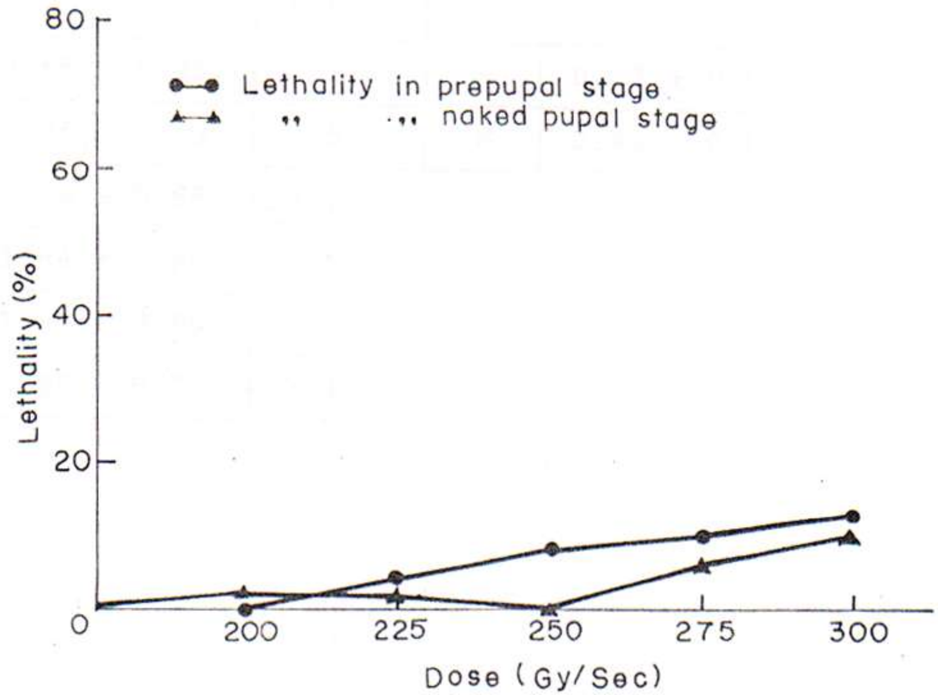


Figure XVIII

LEGENDS

Figure legends:

Figure I and II: Effect of gamma radiation treatment on growth and development of 12 hr old Ist instar larvae of tasar silkworm, A. proylei. Note the difference in size of larvae and cocoon. Bar=5mm.

Figure III:Mortality curve up to maturation of larvae of post-irradiated Ist instar (12hr after hatching) with different doses of gamma radiation in the tasar silkworm, A. proylei.

Figure IV:Mortality curve up to maturation of larvae of post-irradiated IInd instar (12hr after Ist moult) with different doses of gamma radiation in the tasar Silkworm, A. proylei.

Figure V:Mortality curve up to maturation of larvae of post-irradiated IIIrd instar (12hr after IInd moult) with different doses of gamma radiation in the tasar silkworm, A. proylei.

Figure VI:Mortality curve up to maturation of larvae of post-irradiated IVth instar (12hr after IIIrd moult) with different doses of gamma radiation in the tasar silkworm, A. proylei.

Figure VII:Mortality curve up to maturation of larvae of post-irradiated Vth instar (12hr after IVth moult) with different doses of gamma radiation in the tasar silkworm, A. proylei.

Figure VIII:The effect of different doses of ^{60}Co gamma radiation on larval durations of Ist instar larvae (12hr after hatching) in the tasar silkworm, A. proylei.

Figure IX:The effect of different doses of ^{60}Co gamma radiation on larval durations of IInd instar larvae (12hr after Ist moult) in the tasar silkworm, A. proylei.

Figure X:The effect of different doses of ^{60}Co gamma radiation on larval durations of IIIrd instar larvae (12hr after IInd moult) in the tasar silkworm, A. proylei.

Figure XI:The effect of different doses of ^{60}Co gamma radiation on larval durations of IVth instar larvae (12hr after IIIrd moult) in the tasar silkworm,A. proylei.

Figure XII:Comparative studies of different ^{60}Co gamma radiation doses on Vth instar larvae (12hr after IVth moult) of the tasar silkworm, A. proylei on larval durations.

Figure XIII:Effect of different doses of ^{60}Co gamma radiation to Ist instar (12 hr after hatching) larvae on total larval period of the tasar silkworm, A. proylei.

Figure XIV:Effect of different doses of ^{60}Co gamma radiation on IInd instar (12 hr after 1^{st} moult) larvae on total larval period of the tasar silkworm, A. proylei.

Figure XV:Effect of different doses of ^{60}Co gamma radiation to IIIrd instar (12 hr after IInd moult) larvae on total larval period of the tasar silkworm, A. proylei.

Figure XVI: Effect of different doses of ^{60}Co gamma radiation to IVth instar (12 hr after IIIrd moult) larvae on total larval period of the tasar silkworm, A.proylei.

Figure XVII:The lethality in percent in prepupal and naked pupal stages of IVth instar larvae (12 hr after IIIrd moult) of the tasar silkworm, A. proylei treated with different doses of ^{60}Co gamma radiation.

Figure XVIII:The lethality in percent in prepupal and naked pupal stages of Vth instar larvae (12 hr after IVth moult) of the tasar silkworm, A. proylei treated with different doses of ^{60}Co gamma radiation.