Journal of Experimental Sciences 2011, 2(9): 31-35
ISSN: 2218-1768
www.scholarjournals.org

journal of experimental sciences

www.jexpsciences.com

JES-Physical Sciences

Radiation Studies on the Commercial Parameters of the Tasar Silkworm, *Antheraea proylei* (Lepidoptera)

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Article Info

Article History

Received : 06-05-2011
Revised : 11-08-2011
Accepted : 12-08-2011

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Abstract

Commercial parameters namely silk ratio (SR), shell weight, silk yield, effective rate of rearing (ERR), cocoon weight, double cocoon formation, formation and frequency of naked pupae etc. were studied in irradiated tasar silkworm, *Antherea proylei*. The silk parameters were found to be decreasing as the dose is increased, but at low doses vigor is obtained as in treatment with 200 Gy in IVth instar.

Key Words: Radiation,paramiters,silkworm

Introduction

The silkworm cannot be separated from the economic growth of mankind. Exhaustive researches have been conducted on the improvement of this insect and there is still a lot to be done regarding the improvement of silk production. Ionizing radiations such as X-rays and gamma rays are known to widen the genetic variability, enabling the breeder to step up selection for the quantitative characters and are extensively used in crop and animal improvement programs. when studying the effect of gamma radiation on the eggs of 3 polyvoltine strains (Hosa Mysore, C. Nichi and MY1 (sex-limited)) of *Bombyx mori* found that among the treated generation, Hosa Mysore was found to be the most sensitive, followed by C. Nichi and MY1 (sex-limited) affecting the economic characters and suggested that irradiation may be a useful tool for exploring genetic variation in silkworms .In the present study, an attempt is made to study the effect of radiation on the economic parameters of the silkworm.

Materials and Methods

Larvae of equal sizes of 50 individuals and three replicates were taken for each dose treatment in each instar. The larvae of different instars were treated with 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. The twigs were changed two times a day and the water was changed every day. The excreta and pieces of leaves lying on the bottom of the net were cleaned every day to protect from fungal attack.

Observation

The larvae were observed twice a day. The number of death larvae in each replicate after treatment and behavior of moulting were noted. The nature of death, food intake and other behaviors were also noted. Number of cocoons formed was noted. Weights of green cocoons and dry shells were also taken for studying the silk parameters.

Effect of gamma ray exposure on silk production in A. proylei:

The silk ratio (SR), silk yield and effective rate of rearing were studied by rearing the worms on six different dose treatments of the two critical instars - IVth and Vth. The other instars (Ist to IIIrd) were not taken up for this study because, in earlier instars, the mortality was high and so unsuitable for such studies. The parameters studied regarding silk production are defined below

Silk percentage or silk ratio (SR):

This character is measured as the ratio of the shell weight to total cocoon weight expressed in percentage. The silk ratio was calculated by the following formula

$$\text{Silk Ratio(SR)} = \frac{\text{shell weight}}{\text{cocoon weight}} \times 100$$

Shell weight:

It is an important commercial character. It measures the silk content present in a cocoon.

Silk yield:

It is also an important commercial character. It measures the total silk content of cocoons harvested out of 100 larvae reared.

Effective rate of rearing (ERR):

This character measures the rearing performance of a line and is calculated on the basis of the proportion of cocoons harvested to total number of worms mounted expressed in percentage as follows-

$$\text{ERR}=\frac{number\ of\ cocoons\ harvested}{number\ of\ worms\ reared}\times 100$$

Cocoon weight:

This character reflects the vigor of the larvae and is calculated in terms of the weight of the cocoon with pupa.

Double cocoon formation:

The metamorphosis of two larvae and rarely three, into pupae, in one and the same cocoon is referred to as double cocoon formation

Formation and frequency of naked pupae:

The so-called naked pupae are those which cannot form or complete the cocoon formation. Then they transform into incapable (because they are incapable of completing the cocoon formation) larvae, which is similar to prepupal larvae inside the cocoon. Finally they transform into pupae outside the cocoon that is why they are called naked pupae (i.e. pupae without the covering). Then they finally develop limb buds – the first sign of transformation into a moth. But they cannot fully transform into an adult moth and cannot come out of the pupal case and after about 6-10 days in the miniature moth stage, they usually die.

Results

Naked pupae

The so called naked pupae characterized by pupae without cocoon shell that the larvae cannot form silk threads but transform into pupae and then into miniature moth that they form wing and limb buds, cannot complete the process. The radiation might have damaged the silk glands or may be the silk protein cannot be synthesized due to mutations in the synthetic machinery. Because it occurs at high doses only.

Naked pupae were not formed in Ist, IInd and IIIrd instar treatments because the larvae cannot withstand the high doses of radiation and they usually die before spinning. In IVth instar treatments, it was formed in frequencies of 4%, 8%, 2%, 4% and 8% in 150, 175, 200, 225 and 250 Gy treatments respectively. And in Vth instar treatments, it was formed in the frequencies of 2%, 2%, 0%, 6% and 10% in 200, 225, 250, 275 and 300 Gy treatments respectively.

Double cocoon formation:

2% of the individuals in 275 Gy treatments formed double cocoons. Green cocoon weight was 3.96 gm and dry shell weight was 0.51 gm. The combination of sex was male and female. It was observed only in Vth instar treatments.

Green cocoon weight and shell weight:

This character were studied in IVth and Vth instar treatments only because in early instar treatments, the larvae cannot withstand the high doses of radiation and they usually die before spinning. The green cocoon weights and shell weights in controlled and treated samples were taken and the mean values and standard deviations were also calculated and tested for significant difference using t-test. Thus the green cocoon weights in 0, 150, 175, 200, 225 and 250 Gy treatments were found to be 3.55±0.85, 2.84±0.40, 2.31±0.65, 1.73±0.39, 2.98±0.44 and 2.47±0.56 gm respectively whereas cocoon shell weights were 0.27±0.06, 0.26±0.06, 0.23±0.07, 0.19±0.04, 0.25±0.04 and 0.24±0.11 gm respectively (Table I) in IVth instar treatments. Regarding green cocoon weights, the treatments were found significantly lower than controlled value in 150, 175, 200 and 250 Gy treatments and no significant in 225 Gy treatments. Whereas the cocoon shell weights were significantly lower than controlled value in 200 Gy treatments while no significant in 150, 175, 225 and 250 Gy treatments.

In Vth instar treatments, the green cocoon weights were found to be 3.55±0.85, 2.85±0.60, 2.79±0.65, 1.84±0.56, 1.92±0.68 and 1.95±0.78 gm respectively in 0, 200, 225, 250, 275 and 300 Gy treatments respectively (Table II) and cocoon shell weights were 0.27±0.06, 0.42±0.05, 0.37±0.05, 0.32±0.08, 0.37±0.08 and 0.32±0.06 gm respectively. The green cocoon weights were found significantly lower from controlled value whereas the cocoon shell weights were significantly higher in 200-275 Gy treatments, but not significant in 300 Gy treatments. Thus gamma ray treatments were found to be useful in Vth instar larvae regarding cocoon shell weight.

Table I: Weight (in gm) of green cocoon and cocoon shell of gamma rays treated 12 hr old

Dose regimen	Green Cocoon wt (gm)± standard deviation	Cumulative difference (CD.) at 5%	Level of Significance	Cocoon shell (gm) ± standard deviation	Cumulative difference (CD.) at 5%	Level of significance
0	3.55±0.85	Nil	Nil	0.27±0.06	Nil	Nil
150	2. 84±0.40	2.29	S	0.26±0.06	0.5	NS
175	2.31±0.65	3.1	S	0.23±0.07	1.33	NS
200	1.73±0.39	3.5	S	0.l9±0.04	2.67	S
225	2.98±0.44	0.95	NS	0.25±0.04	0.67	NS
250	2.47±0.56	2.12	S	0.24±0.1l	0.75	NS

IVth instar larvae of tasar silkworm, ***A. proylei.***

Table II Weight (in gm) of green cocoon and cocoon shell of gamma radiation treated with 12 hr old Vth instar larvae of tasar silkworm, ***A. proylei.***

Dose regimen	Green Cocoon wt (gm) ± standard deviation S	Cumulative difference (CD.) at 5%	Level of Significance	Cocoon shell (gm) ± standard deviation S	Cumulative difference (CD.) at 5%	Level of Significance
0	3.55±0.85	Nil	Nil	0.27±0.06	Nil	Nil
200	2.85±0.60	2.5	S	0.42±0.05	5.00	S
225	2.79±0.65	2.11	S	0.37±0.05	3.33	S
250	1.84±0.56	4.5	S	0.32±0.08	2.5	S
275	1.92±0.68	3.88	S	0.37±0.08	3.33	S
300	1.95±0.78	3.4	S	0.32±0.06	1.67	NS

Table III Silk ratio (SR) and effective rate of rearing (ERR) of the IVth instar tasar silkworm, ***A. proylei*** reared on six dose regimen.

Dose regimen	Silk ratio (SR %) Mean of male + female cocoon ± standard deviation	Cumulative difference (CD.) at 5%	Level of Significance	Percentage effective rate of rearing (ERR)
0	8.99 ± 4.75	Nil	Nil	93
150	9.60 ± 1.49	0.402	NS	34
175	11.26 ± 5.25	1.207	NS	28
200	12.34 ± 3.48	1.570	NS	14
225	11.79 ± 3.12	1.440	NS	13
250	9.38 ± 3.58	0.170	NS	12

Table IV Silk ratio (SR) and effective rate of rearing (ERR) of the Vth instar tasar silkworm, ***A. proylei*** reared on six dose regimen.

Dose regimen	Silk ratio (SR %) Mean of Male + Female cocoon ± standard deviation	Cumulative difference (C.D.) at 5%	Level of Significance	Percentage effective rate of rearing (ERR)
0	8.99 ± 4.75	Nil	Nil	97
200	14.93 ± 4.43	3.48	S	98
225	13.66 ± 2.40	2.47	S	61
250	14.84 ± 3.79	3.10	S	62
275	19.80 ± 5.99	4.50	S	59
300	20.24 ± 6.75	4.31	S	26

Silk ratio (SR) and effective rate of rearing (ERR):

The SR and ERR values which were good estimators of commercial value in silkworms, were calculated separately for each individual and the mean was calculated for each treatment. Standard deviations were also calculated. Thus the mean SR values were found to be 8.99±4.75, 9.60±1.49, 11.26±5.25, 12.34±3.48, 11.79±3.12 and 9.38±3.58 respectively in 0, 150, 175, 200, 225 and 250 Gy treatments (Table III) in IVth instar treatments and the mean ERR values were 50, 34, 28, 14, 13 and 12 respectively. The mean SR values were tested for significant difference using t-test. It appears from the table that the values of silk ratio were not significantly different at 5% level of confidence between controlled and treated animals at the IVth instar. The ERR values were 93, 34, 28, 14, 13 and 12 in 0, 150, 175, 200, 225 and 250 Gy treatments respectively. Whereas the mean SR values were found to be 8.99±4.75, 14.93±4.43, 13.662±.40, 14.84±3.79, 19.80±5.99 and 20.24±6.75 gm respectively in 0, 200, 225, 250, 275 and 300 Gy treatments respectively in Vth instar treatments (Table IV). All the treatments from 200 to 300 Gy were found to be significantly different from the controlled value and the values were higher in treated samples. So gamma ray can induce useful effects in the silk ratio of the Vth instar larvae of *A. proylei*. The ERR values were 97, 98, 61, 62, 59 and 26 in 0, 200, 225, 250, 275 and 300 Gy treatments. The ERR value was higher in 200 Gy treatment. Thus among the treatments in the present study 200 Gy is the most effective in Vth instar treatment.

It was observed that, in Ist instar treatments, there was no formation of naked pupae; 6% of the larvae in 50 Gy treatment died in the prepupal larval stage which is the non moving, non feeding and constricted stage after maturation of larvae, which will moult to pupae in normal cases. 4% of the larvae in 75 Gy treatments died in the prepupal larval stage and none in the 100 Gy treatments. The silk output was comparatively low, only some threads could be seen on the leaves. Food intake was lower in greater dose treatments. The larvae after sometime had stopped eating and became dull in movement and died after some period of radiation sickness. Excretion was found negatively correlated with the radiation dose. As higher dose treated animals consumed less food, their excreta was also less in quantity. The excreta were sticky and mucus like secretion, sometimes produced in chains. In the larval deaths, not in the moulting process, there was an oozing of haemolymph through the mouth and vomiting of greenish liquid. Of course, the frequency of such deaths was comparatively lower than that of moulting death.

In IInd instar treatments, growth and development gradually decreased with the gradual increase of dose. There was no formation of naked pupae and the silk output was comparatively low. Most of the larvae died after making some threads not in the form of a cocoon. 12% of the larvae died in the prepupal larval stage in 100 Gy treatment, only 2% in 125 Gy and none in 150 Gy treatments because of enhancement of death due to radiation induced abnormalities. The nature of death was similar to that of treated Ist instar.

In IIIrd instar treatments also, the nature of manifestation of behavior after treatment on growth, movement, feeding, excreta, moulting process, gradual process of senescence due to radiation sickness and silk output were similar to those of earlier instar treatments. 4% of the larvae formed constricted prepupal larval stage and died before moulting to pupae in 150 Gy treatments and none in the 200 Gy treatments.

In IVth instar treatments; although the nature of radiation sickness was similar to those of earlier instar treatments, they become more radioresistant.

In Vth instar treatments, the amount of food intake was higher in 200 Gy treatments than the controlled and other dose treatments. The 200 Gy larvae were active in comparison with other dose treatments; as a result, the amount of excreta was higher in it. In 200 Gy treatments, 20% of the individuals were found still alive and healthy when dissected for weighing of the dry shell. The larvae as a whole in treated samples were not dull as was the case in earlier instar treatments. The movements were also normal. Of course, in 300 Gy treatments, the dullness and slow movement could be noticed after some days of treatment.

Again the LD_{50} values could be considered which gradually increases 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 which indicates gradual increase in radioresistance of the different metamorphological groups.

Discussion

The present study aims at evaluating the effects of gamma radiation on the commercial characters of the economic hybrid species of tasar silkworm, *Antheraea proylei* and finally to draw the degree of radioresistance for commercial utilization.

Attempts were made by many investigators to study the sex combinations in double cocoons of mulberry silkworm, *Bombyx mori* (Rao and Rao, 1961; Kumararaj, 1968; Tayade, 1982-83; Narayanaprakash et. al., 1984; Govindan and Narayanaswamy, 1985). On an average, the occurrence of male-female combination was stated to be highest (Rao and Rao, 1961; Kumararaj, 1968; Narayanaprakash et. al., 1984) followed by female-female and male-male combinations (Kumararaj, 1968). In the double cocoon formed in the present experiment with 275 Gy treatment on 12 hr old Vth instar tasar silkworm, *A. proylei*, the combination was male-female.

Evaluation of silk parameters to estimate the silk production of different irradiated individuals and populations show declination of silk output from lower to higher doses. This might be due to stunted development of silk gland which ultimately might have led to low secretion of silk protein. The silk threads were in flimsy cocoon formation in spontaneous mutants leading to developmental abnormalities in the posterior silk gland accompanied by reduced fibroin and/or sericin production and poor cocooning in *B. mori* (Suzuki, 1977). The effects of UV irradiation on the survival of *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae) were investigated by Snigdha Mohan and Dinesh Kumar (2010). UV irradiated nymphs exhibited a significant decrease in survival with increasing exposure time. They showed delayed moulting into adults, which also showed significantly decreased survival. In the present study, poor cocooning was, of course, observed, but the quality of silk was not inferior. Ovarian development of *Tenebrio molitor* showed dose dependant gamma radiation disruption (Menon and Nair, 1972). A dose of 2 Krad of beta

radiation permanently inhibits oothecal formation in *Periplaneta americana* (Wharton and Wharton, 1957). Effects of some biologically important substances (aqueous solutions of ascorbic acid, biotin, bovine serum albumin, theophylline, 5-HT and c-AMP) on some economic characters of silkworm, *Bombyx mori* L. were studied (Tapan andDatta, 2003). None of the biologically active chemicals exhibited any major effect on shell weight and SR% while all improved ERR%. The cocoons spun by PM x CSR2 fed on mulberry grown by supplying with different types of organic manures exhibited notable influence on cocoon traits (Shashidhar et. al., 2009). Interestingly the results in the present study showed that 200-300 Gy treatments of gamma ray on 12 hr old Vth instar larvae of *A. proylei* leads to significantly higher silk ratios than corresponding control populations. This may be due to some kind of positive mutations induced by gamma radiation.

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