CHAPTER - II

MATERIALS AND METHODS
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The present investigations were undertaken to study of some CSR hybrid races of silkworm *Bombyx mori* L. and seasonal impact on their rearing performance. Influence of mulberry varieties, disease prevalence, fortification study and weeds of mulberry garden, etc. were also studied.

Location

The studies were carried out at Sericulture Research Unit, Zoology Department, Dr. Babasaheb Ambedker Marathwada University campus, Aurangabad (Maharashtra) (Plate-1). Aurangabad is situated on 19\(^0\) 52' North Latitude and 75\(^0\) 18' East Longitude. The mean annual rainfall is about 750-850 mm. The mean daily maximum temperature varies from 30 \(^\circ\)C in December to 45 \(^\circ\)C in May. The minimum temperature varies from 11 \(^\circ\)C in winter and 25-27 \(^\circ\)C in summer. The mean relative humidity ranges from 30-90 %, as it is observed during study period (2004-06).

2.1 Materials used

2.1.1 Seed Stock

The seed stock of three promising silkworm hybrids was obtained from National Silkworm Seed Project, near Madiwala Police Station, Madiwala, Bangalore- 560068, Karanataka State, India.
The experiment on performance of hybrid races and seasonal impact on their rearing were carried out with following promising silkworm hybrids.

CSR2 X CSR4
CSR4 X CSR2
PM X CSR2

The experiments influence of mulberry varieties, disease prevalence and fortification studies and biological characteristics of silkworm *Bombyx mori* were conducted with CSR2 X CSR4 hybrid.

2.1.2 Rearing Equipments

Following equipments were used for rearing the silkworms,

i) Rearing trays.

ii) Rearing stand.

iii) Chopping board.

iv) Chopping knife.

v) Chop sticks (used for giving space for young age, larval handling in diseased cases).

vi) Feathers (used to brush newly hatched worms).

vii) Cleaning nets.

viii) Mountages (plastic).

ix) Paraffin paper, polythene sheet and foam pads to maintain humidity in rearing beds.

x) Formalin/Formaldehyde.

xi) ‘Vijetha’- bed disinfectant.

xii) 0.5% Slaked lime.

xiii) Muslin cloth – used for dusting ‘vijetha’.

xiv) Humidifier.
Polymeter (used to record temperature and humidity).

Electric heater and other minor equipments necessary during rearing.

2.2 Rearing Method

The rearing of silkworm was conducted as per the technology suggested by Krishnaswami, (1978) and Hiware, (2001). The rearing of silkworm hybrids was undertaken with the use of well grown mulberry plantation of different mulberry varieties.

The rearing house (Plate-2a) and all the rearing appliances were disinfected with Formalin solution (2-4 %) to make them free from pathogens before rearing. Paper sheet of disease free layings (dfls) of silkworm hybrids were obtained from NSSP, Bangalore and were incubated at of 25 ± 1°C and 75 ± 5 % relative humidity. The egg sheets were spread out in a single layer in rearing trays and covered with paraffin paper. Wet foam rubber pads were kept all around the egg sheets to ensure the required humidity for incubation. The trays containing egg sheets were stored in cool place in rearing house (Plate-2b). On attaining the blue egg stage, the egg sheets were placed into a card board box and covered by black piece of cloth and left undisturbed for uniform growth of embryo, after which the egg were exposed to bright light for one hour for uniform hatching immediately after hatching fresh tender mulberry leaves cut into size 0.5 to 1.0 sq. cm were sprinkled on worms. When all the larvae crawled over the sprinkled leaves. The leaves along with the worms were gently brushed on the polythene sheets spread in the rearing trays (Plate-3a).
Plate - 2

(a) Rearing House, Sericulture Research Unit, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

(b) Shelf rearing of silkworm with wooden trays.
Soon after brushing rearing bed was made with more chopped leaves and covered with polythene sheet. Clean wet foam pads were placed around the rearing bed to ensure required humidity. The rearing room temperature was maintained at about $27^0C \pm 1$. The trays were piled up one above other in the form of box to maintain temperature to conserve leaf moisture and to ensure vigorous larval growth. Tender mulberry leaves with more moisture content were fed for feeding the worms in the initial stages of rearing. The worms were fed four times a day with chopped leaves for feeding first instar larvae highly nutritious top two full blown leaves as mulberry immediately below the growing bud were plucked and fed fresh. Care was taken to distribute the worms uniformly and to maintain the space in the rearing bed. Everyday morning before feeding the beds were spread to dry the leaves and to enable the larvae to crawl on the surface of fresh leaves. This helped in maintaining hygiene and micro climate of bed. When the worms started setting for first moult the polythene sheet cover and wet foam pads were removed. Feeding was reduced during the period. During moult ing the rearing bed was kept dry and undisturbed. The worms required about 24 hrs. to come out of first moult. When the worms were ready for resumption of feeding the bed disinfectant Vijetha was taken in a thin cloth and dusted all over the worms as per the schedule mentioned by Kawakami, et al., (2003) per 100 dfls. Feeding was resumed 30 minutes later.

Clean nylon bed nets of required mesh size were spread on the bed and chopped tender mulberry leaves spread on it. In short time all the larvae crawled on the leaves over the net and were lifted
Plate - 3

(a) Brushing with Leaf feeding.

(b) Single cocoon assessment.
along with net. After removing the bed waste, the worms were placed back in the trays. The wet foam pads were placed in position and the trays were covered with polythene sheet. The feeding was continued four times a day. The rearing beds were cleaned daily in morning expanding the size of bed corresponding to growth of larvae. When larvae settled for 2\textsuperscript{nd} moult again foam pads and polythene covers were removed to unnecessary moisture in the tray. When worms completely settled for moult, feeding was stopped. In about 24 hrs time all the larvae came out of 2\textsuperscript{nd} moult. The bed disinfected with ‘Vijetha’, the larvae were fed with tender mulberry leaves and beds were cleaned as described earlier. As per requirement space was provided in 3\textsuperscript{rd} instar the polythene sheet was spread and the wet foam pads were not used due to less requirements of relative humidity i.e. below 80 %. The leaves with medium maturity were feed. The rearing beds were cleaned daily in morning from third instar onwards. In the third instar larvae were fed for 3 to 3.5 days after which they settled for moult. Following the regular procedure, the feeding was resumed and required spacing was provided after 24 hours. The fourth instar took 4 to 4.5 days followed by 4\textsuperscript{th} moult (about 30 hrs). The duration of 5\textsuperscript{th} instar was normally 6 to 7 days and the larvae started to spin cocoons by the end of this stage. On the 4\textsuperscript{th} day of 5\textsuperscript{th} instar the bed was disinfected with ‘Vijetha’ once more. During 5\textsuperscript{th} instar larvae were fed with fully matured mulberry leaves and for the last two days coarse leaves were used for feeding at the end of 5\textsuperscript{th} instar larvae release wet faecal matter, shrink in size, body becomes translucent and start crawling in the bed with raised head. These are indications that larvae were ready for spinning the cocoon. They were individually
picked up and transferred to mountages for spinning the cocoon. When about 70-80% worms were mounted, the remaining worms were mounted at once. The Larvae were left undisturbed till 6th day of spinning after which cocoons were harvested.

2.3 Study of some CSR hybrid races of silkworm *Bombyx mori* L. and seasonal impact on their rearing performance

2.3.1 Experimental details

I) Design : Completely Randomized Design (CRD)  
II) No. of replications : 3  
III) Mulberry variety : V1  
IV) Number of hybrids : 3  
    CSR2 X CSR4  
    CSR4 X CSR2  
    PM X CSR2

2.3.2 Method of recording observations

Observations were recorded on hatching percentage by egg sheets and other following parameters on ten randomly selected larvae / cocoons of silkworm hybrids from each race.

2.3.2.1 Hatching Percentage

This denotes the number of larvae that successfully come out from the silkworm egg the hatching percentage is calculated as follows
Hatching (%) = \frac{\text{Total number of Larvae hatched}}{\text{Total number of egg}} \times 100

2.3.2.2 Larval duration (days)

The mean larval span was recorded from hatching to per spinning stage (including moulting duration) and computed in days.

2.3.2.3 Larval weight (g)

Mean larval weight was recorded in grams for ten randomly selected five day old 5\textsuperscript{th} instar larvae from each replication and hybrid.

2.3.2.4 Single cocoon weight (g)

The cocoon weight (g) was calculated as average weight of ten cocoons taken at random each replication.

\[
\text{Cocoon weight} = \frac{\text{Weight of cocoon (g)}}{\text{Number of cocoon}}
\]

2.3.2.5 Single cocoon shell weight (g)

The shell weight (g) calculated as average weight as 10 shells taken at random from each replication.

\[
\text{Cocoon shell weight} = \frac{\text{Weight of shell (g)}}{\text{Total number of shell}}
\]

2.3.2.5 Shell ratio percentage (%)

The cocoon shell ratio was determined by dividing the cocoon shell weight by cocoon weight. It is expressed in percentage.
Cocoon shell ratio (\%) = \frac{\text{Weight of cocoon shell (g)}}{\text{Weight of cocoon (g)}} \times 100

2.3.2.7 Filament length (m)

The length of filament reeled from single cocoon is measured in meter. Ten cocoon were collected randomly from each replication and reeled individually. An average of ten cocoon is recorded.

2.3.2.8 Filament weight (g)

The weight of filament was measured in gram which reeled from individual cocoon.

2.3.2.9 Denier

This denotes the thickness of the filament. Nine thousand meters of silk filament weighing 1gm is considered as 1 denier. The reeled silk was dried and weight was taken for calculation.

\text{Denier} = \frac{\text{Weight of raw silk reeled (g)}}{\text{Weight of raw silk reeled (m)}} \times 9000

The above post cocoon characters has been assessed by single cocoon assessment (Plate-3b).

2.4 Influence of different mulberry varieties (V1, M5 and S1635) on silkworm rearing.

The experiment was conducted during post rainy (Aug.-Sept.) season of the year 2004-2005 and 2005-06.
2.4.1 Experimental details

A promising bivoltine x bivoltine mulberry silkworm hybrid CSR2 X CSR4 was used as test material.

- **Design**: Completely Randomized Design (CRD)
- **No. of replications**: 3
- **Mulberry varieties used**: 
  - V-1 (Victory 1) **(Plate-4)**
  - M-5 (Kanva 2)
  - S1635

A popular mulberry silkworm hybrid CSR2 x CSR4 was used for observing the influence of different mulberry varieties (V1 M5 and S1635) on silkworm rearing. The experiment was commenced from 5th instar as this is the stage in which the silkworm consumes more than 80% of the total feed. After the 4th moult larvae were grouped into three groups of 100 healthy larvae, with three replicas and reared on V1, M5 and S1635 mulberry varieties separately during post rainy season (September) of the year.

2.4.2 Observations recorded

Observations on 5th instar larval duration, larval weight, cocoon weight, cocoon shell weight, pupal weight, shell ratio percentage, filament length, filament weight and denier of the filament were recorded.
Plate - 4

Field View of mulberry varieties - V1, M5 and S1635

(a) V1 Mulberry variety.

(b) M5 Mulberry variety.

(c) S1635 Mulberry variety.
2.5 Disease Prevalence

During the silkworm rearing different diseases muscardine, grasserie and flacherie occurred. The infected larvae of respective disease were collected, number was recorded and the prevalence was calculated by using the formula.

\[
\text{Disease prevalence (\%)} = \frac{\text{No. of diseased larvae}}{\text{Total number of larvae}} \times 100
\]

2.6 Fortification studies and biological characterization of Silkworm.

2.6.1 Effect of plant extracts on economic traits of silkworm hybrid CSR2 x CSR4

Experimental details

i) Design : Completely Randomized Design (CRD)

ii) No. of Replication : 3

iii) Mulberry variety : V1

iv) Number of treatments : 4

\[T1 = Phyllanthus niruri\]
\[T2 = Tephrosia purpurea\]
\[T3 = Phyllanthus emblica\]
\[T4 = Phyllanthus amarus\]

The disease free laying of CSR2 x CSR4 was used for the evaluation of fortification studies and biological characteristic of silkworm. The experiments were conducted by taking randomly
fresh 4\textsuperscript{th} moult parsed 5\textsuperscript{th} instar larvae in five groups each containing 50 larvae with three replicas. For fortification the fresh parts of plant, *Phyllanthus niruri* (plants without root), *Tephrosia purpurea* (root of plants), *Phyllanthus emblica* (fruit) and *Phyllanthus amarus* (plant without root) were procured from the Dr. Babasaheb Ambedkar Marathwada University campus Aurangabad and different parts as shown in parentheses were used to prepare the test solution.

The test solution were prepared by crushing 25g of the plant material by using 100 ml distilled water filtered through muslin cloth and the filtrates was used as stock solution, kept in refrigerator. The quantity of feed given to the all groups with 40 g of matured mulberry chopped leaves for each feed and 4 feedings per day were provided. One group was kept control giving the first feeding by using non treated only distilled water for first feed but the experimental group was given first feed sprinkled, mixed with the 4 ml of test solution till the larvae went on spinning.

**Observations recorded**

Observation on the larval weight, mortality of larvae, cocoon weight, shell weight, pupal weight, shell ratio percentage, filament length, filament weight and denier of filament were recorded and the values were compared in between experimental and control groups by showing per cent change over control.

**2.6.2 Effect of certain drugs on economic traits of silkworm hybrid CSR2 x CSR4.**

Experimental details
i) Design : Completely Randomized Design (CRD)

ii) No. of replication : 3

iii) Mulberry variety : V1

iv) Number of treatments : 3

   T1 = Chelidonium
   T2 = Phytolacca berry
   T3 = Nux vomica

The productive bi x bivoltine hybrid CSR2 x CSR4 was used for evolution of effect of above drugs on biological characters of silkworm.

The experiment was conducted by taking randomly just 4th moult passed i.e. 5th instar larvae in four groups. For each group i.e. one control and three experiments 50 larvae were taken in three replicates. All the groups were exposing to the trial under same environmental condition.

For fortification the mother tincture of Chelidonium, Nux vomica and Phytolacca berry were procured from local Central Homeopathic Pharmacy Shop, Dalalwadi. Aurangabad, M.S. India. The test solutions were prepared by using 10 ml of drug with 40 ml of distilled water was used as stock solution, kept in refrigerator. The quantity of feed given to the all groups with 40 g of matured mulberry chopped leaves for each feed and 4 feedings per day were provided. One group was kept control giving the first feeding by using non treated only distilled water but the experimental group was given first feed sprinkled, mixed with the 2 ml of test solution till the larvae went on spinning.
Observation recorded.

Observation on the larval weight, mortality of the larvae, cocoon weight, shell weight, pupal weight, shell ratio percentage, filament length, filament weight and denier of filament were recorded and the values were compared in between experimental and control group.

2.7 Studies on the weeds of mulberry garden.

Survey of weeds was made in mulberry garden in three different seasons namely rainy, winter and summer during study period of two years i.e. 2004-05 and 2005-06. Collected weeds were identified and placed under respective families with the help of Flora of Marathwada, Naik V. N., (1998).

The collections of weeds were carried out in mulberry garden of Dr. Babasaheb Ambedker Marathwada University campus, Aurangabad (M.S.).

Statistical analysis

The data were statistically analysed by standard “Analysis of Variance” method (Mungikar, A., 2003). The appropriate Standard Error (S.E.) and Critical Difference (C.D.) at five percent level of probability were calculated for comparing the treatments means. Pooled analysis of the data of two years was carried out as per the method described by Mungikar, A., (2003).

For fortification study the significant difference between control and experimental groups were observed by t - test.