University of Mysore

YUVARAJA'S COLLEGE

(Autonomous)

Mysuru – 570 005

Graduate Course – Semester and CBCS Scheme

A
E – BOOK
FOR
PAPER – DSE 2 LAB

SILKWORM SEED TECHNOLOGY

Editor
Dr. H.B. MAHESHA
Associate Professor
Department of Sericulture
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Editor acknowledges all scientists who have pioneered and contributed information used in this e-book.

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CONTENTS

Experiment No. 1: Morphology of Silkworm Egg and Mounting of 7th, 8th and 9th Day Old Embryo.

Aim: To study the morphology of silkworm egg and prepare the temporary mounting of silkworm embryo.

Requirements:

1. Silkworm eggs and embryos

2. 2% KOH: Dissolve 2 gm of KOH in 80 ml of water and make up to 100 ml.

3. Alcohol: 70, 80, 90 and 100 %.

4. Eosine: Dissolve 1 gm of powder in 90% ethyl alcohol.

Xylene, DPX (Di-N-Butyle Phthalate in Xylene), cavity slides, cover glass *etc*.,

Procedure

To study the morphology of silkworm egg: Take a silkworm egg on a glass slide, observe under a stereosome / dissection microscope and list out the features as described below.

For temporary preparation of embryo mounting:

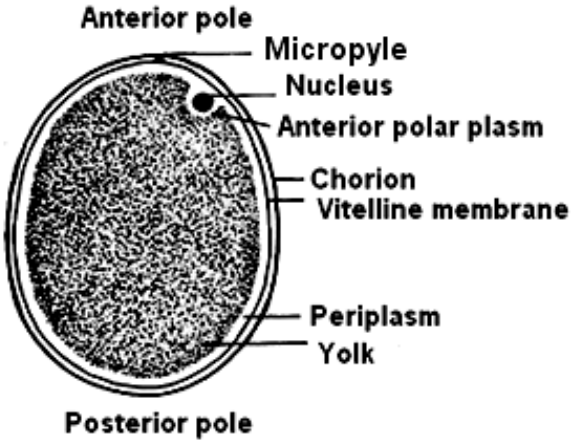
1. Take few embryos in a test tube with 2-3 ml of 2% KOH and boil on a direct flame for one min.
2. Transfer them in to a Petri plate containing cold distilled water and separate the embryos from their shells.
3. Mount the silkworm embryo with a drop of 50 % glycerin on a cavity slide with cover glass.

For permanent preparation of embryo mounting

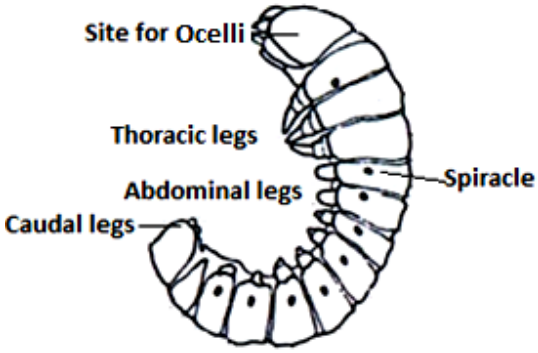
Dehydrate the silkworm embryos by passing through 70 %, 80 % and 90 % alcohol for ten min each. Stain the embryo in alcoholic eosin for 1 min. Again pass through absolute alcohol and xylene for 10 min each. Now mount the embryo with a drop of DPX on a cavity slide with cover glass. Allow the slides for complete drying of DPX.

Structure of silkworm egg

The eggs are ovoid, spherical or ellipsoid in shape and flat on the side by which they are attached to the substratum. There is only one micropyle at the anterior pole. Soon after entrance the micropyle branches into three small canals which reach the inside of the chorion. The sperm enters through the micropyle. The respiratory canals are funnel-shaped tubes which become smaller as they go inward. The air necessary for respiration enters through the respiratory canal and CO_2 is expelled. A thin vitelline membrane is found close to the inside of the chorion and it covers the protoplasm and the yolk. The yolk is concentrated at the centre of the egg so that the cytoplasm is pushed to the surface as a thin layer called periplasm. At the micropylar end the periplasm gets collected in cup like depression on the yolk. This area is called the anterior polarplasm and contains the egg nucleus.

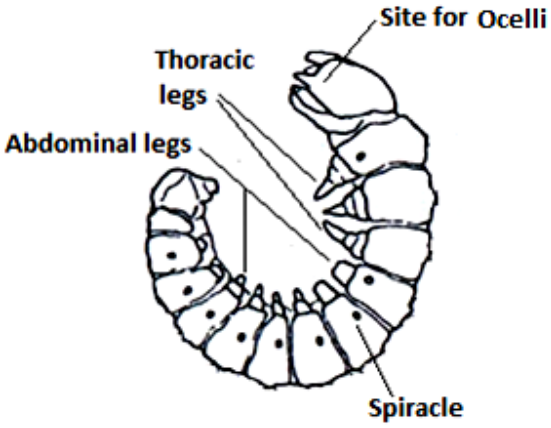


Fifth Day Embryo



After fourth day, the anterior segments of the embryo fuse together and form the head. Thoracic appendages develop and become three segmented legs. The last three segments of the abdomen fuse together so that the abdomen has only nine segments. The embryo now starts to move around as it seeks its correct position in the egg; *i.e.*, the embryo that was in a supine position with its abdomen facing the outer of the egg turns round so that its abdomen faces the inner side. This is called blastokinesis, or involution or revolution of the embryo.

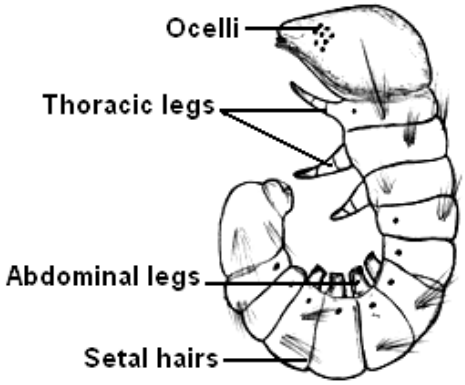
Sixth Day Embryo



After blastokinesis, *i.e.*, on 6th day the embryo continues to develop and the rest of the organs from the ectoderm and mesoderm are formed. The skin, antennae, mouth, thoracic legs, abdominal legs, silk glands, Malpighian tubules, nervous system, endoskeleton in the head ocelli, fore and hind-guts, and the external sexual organs, muscles, dorsal vessel, blood cells, sub oesophageal gland and fat body are formed from the mesoderm. The mid-gut is formed from the

endoderm.

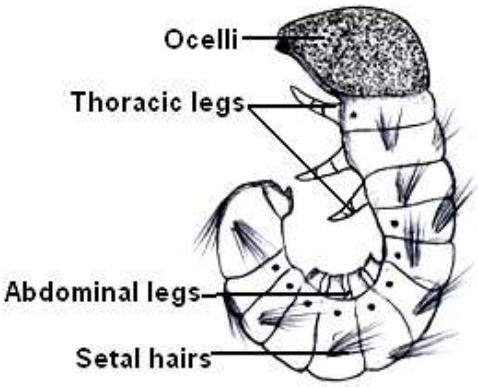
Seventh Day Embryo



Seventh Say Embryo

The segmentation of the body is completed by the end of 7th day of embryonic development. Three pairs of thoracic and five pairs of abdominal legs are well developed. The body segments are well differentiated. The posterior three segments of the abdominal region fused together to look like one segment. There are nine pairs of spiracles placed laterally on either side of the body. They are found on the first thoracic segment and first eight abdominal segments. The bristles are also seen with pale colour.

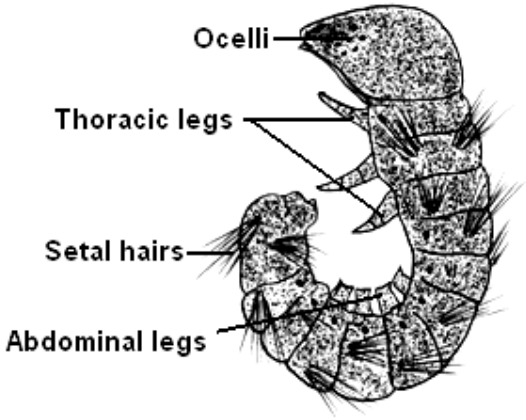
Eighth Day Embryo



In this stage the different organs of the body assumes their clear shape and position within the body. On the body surface setal hairs are prominent. The black pigmentation starts at mouth and head region. Due this pigmentation the head

region of the embryo appears as blue through semi transparent chorion. Hence this stage is known as eye spot stage or pin head stage.

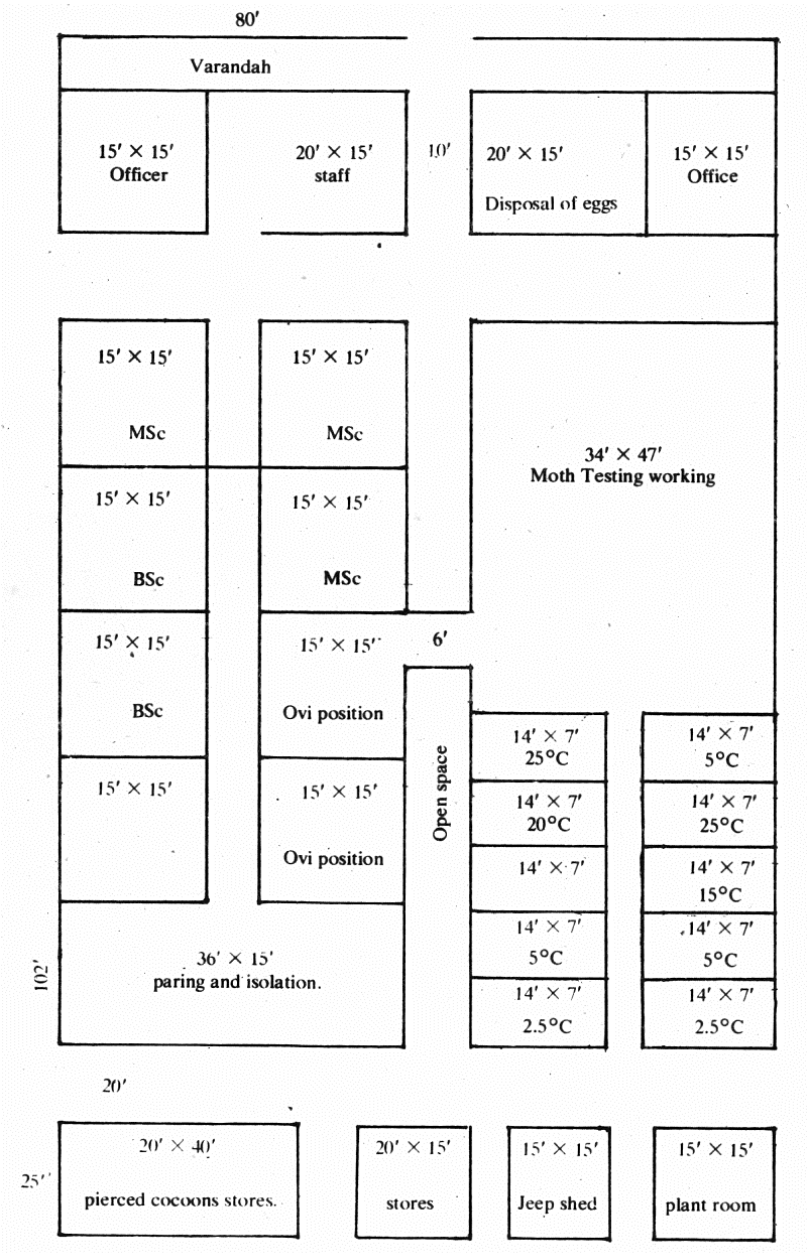
Ninth Day Embryo



The pigmentation initiated at mouth and head region on 8th day will continues to the whole body and becomes dark in colour. However through translucent egg shell it appears as blue hence this stage is known as blue egg stage. At this stage the remaining organs of the developing embryo will be differentiated well and complete the development of all organs. In this stage the embryo should be subjected for black box technique to get uniform and maximum hatching on next day.

Experiment No. 2: Model Grainage Building Plan.

Model Grainage Building Plan: Grainage is a center, where the silkworm seeds are prepared under scientific lines.



Model Grainage Building Plan (15-20 Lakhs DFLs/Annum)

MSc- Pure Mysore Seed Cocoon; BSc- Bivoltine Seed Cocoon

Grainages are generally located in commercial cocoon producing areas to ensure quick supply of silkworm seed to commercial cocoon producers. But they should not be far away from seed areas. The grainage building should have adequate facilities to accommodating the cocoons/pupae of different races and sexes on separate rooms. Rooms for coupling and oviposition should be separate. The rooms particularly, cocoon preservation rooms, pairing room, oviposition, incubation rooms must be provided with facilities to maintain temperature and humidity. Also, facilities to provide darkness and light when needed. Facilities to keep rooms ventilated & clean and for avoiding scales from moths settling in the room must be available. Moth examination laboratory as well as seed processing sections should be isolated. The moth examination rooms must be provided with wider windows and artificial light for examination. Egg processing room must be provided with 3 tier sinks for egg washing and facilities for acid treatment of bivoltines. The incubation room should be away from seed producing wing. A full scale cold storage must be included in the grainage with facilities for storing moths and eggs separately. The provision of a dormitory will increase the efficiency of workers.

Experiment No. 3: Grainage Equipments.

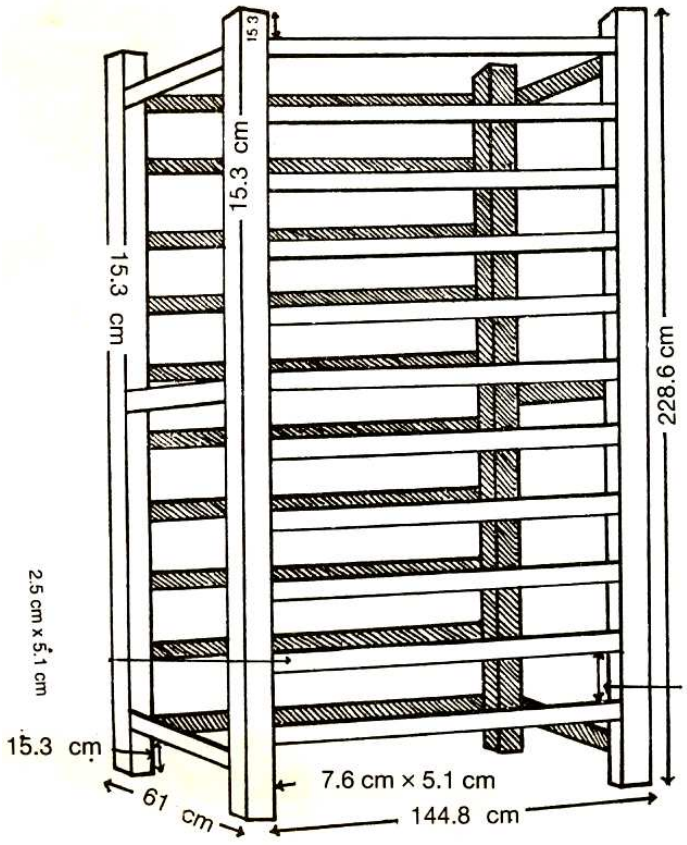
Grainage Equipments

The equipments required for the grainage and their utility are described as follows

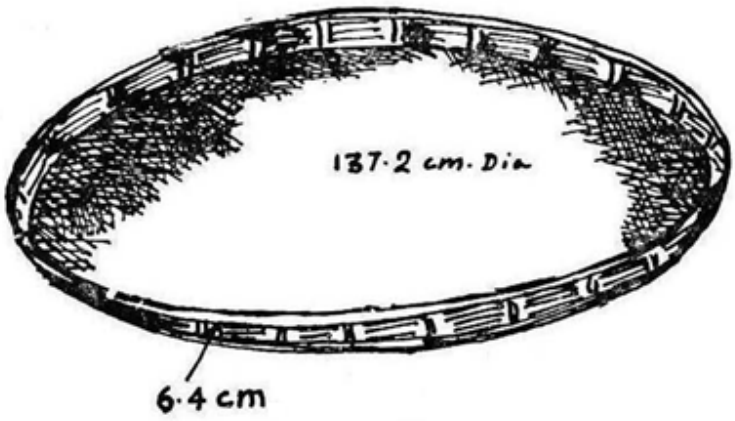
1. **Cocoon Preservation Rack**

   Cocoon preservation racks are made of wood or steel or bamboo and are portable so that it is easy to move them from place to place. The trays are arranged on the shelves and each stand can accommodate ten trays. These are used for keeping the trays containing cocoons and pupae of different races and sexes. The standard length / width of the stand are given with

picture. However, it can be altered depending on the requirement of the grainage.



2. Cocoon Preservation Bamboo Tray

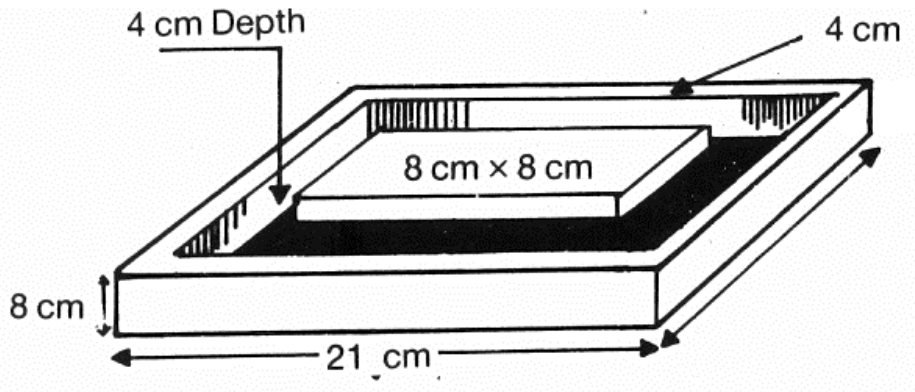


These are used to preserve cocoons. Bamboo trays are economical, light weight, easy to handle and easy for disinfection by smearing with cow dung followed by sun drying. These types of trays are very popular in both Karnataka and West Bengal. The size / diameter is varies according to the requirement and maker.

3. Cocoon Preservation Wooden/PVC tray

The wooden trays are made of light wood and of convenient size for easy handling. Rectangular wooden trays with ply wood bottom are generally used for pairing and oviposition. However, PVC trays are more popular as they are light weight, easy handling and easy for disinfection with any chemical.

4. Antwell



These are made up of either cement or stone blocks or PVC material and the dimensions are given with picture. The legs of the cocoon preservation stands rest on the center of the block an water is poured into the groove to stop the ants

and other wingless or crawling insects climbing on to racks as they are gregarious pests of silkworms/pupae/moths. Each stand leg must rest in a well.

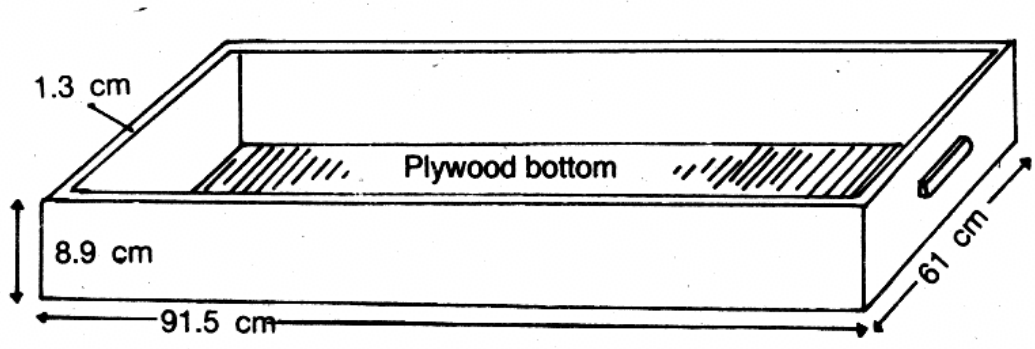
5. Hygrometer

It is one of the important ecological instruments used in recoding atmospheric humidity. The atmospheric humidity is expressed in percentage. In grainages it is used in the cocoon / pupae / moth preservation rooms in general and trays in particular to monitor the atmospheric humidity.

6. Dry and Wet bulb thermometer

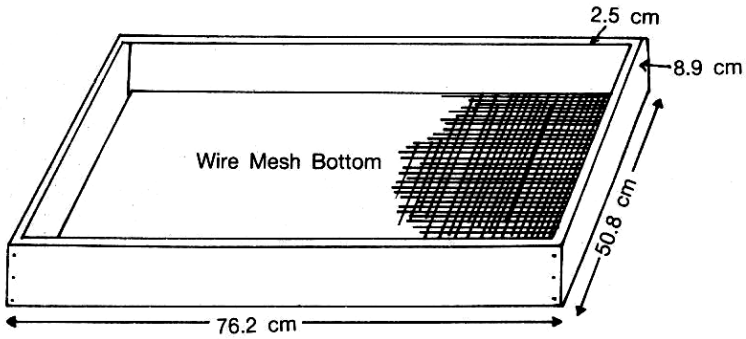
It is also an important ecological instrument and used to record dry as well as wet temperature. By consulting the chart given with the instrument any one can calculate relative humidity of that particular place. In grainages, it is used in cocoon / pupae / moth preservation rooms to monitor both dry temperature and relative humidity.

7. Grainage Tray-Plywood bottom



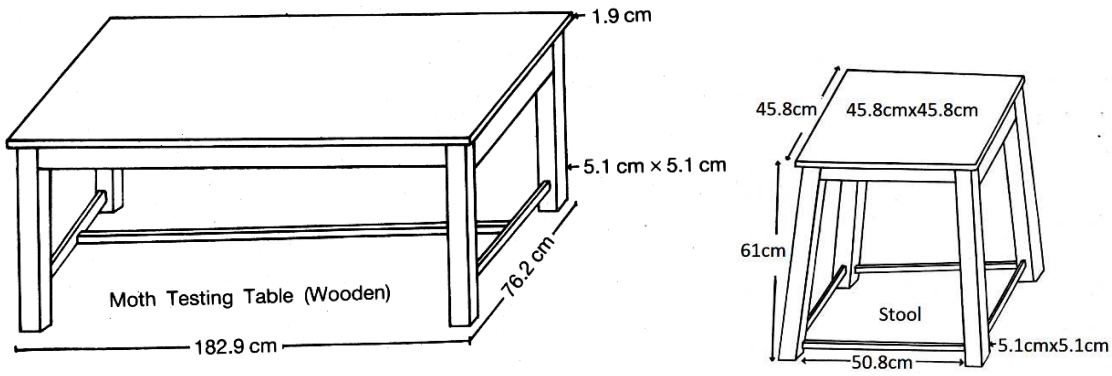
In grainages, different types of trays are used. For preservation of moths, during copulation and oviposition wooden trays with plywood bottom is generally used as it provides smooth, uniform bottom.

8. Grainage Tray wire mesh bottom



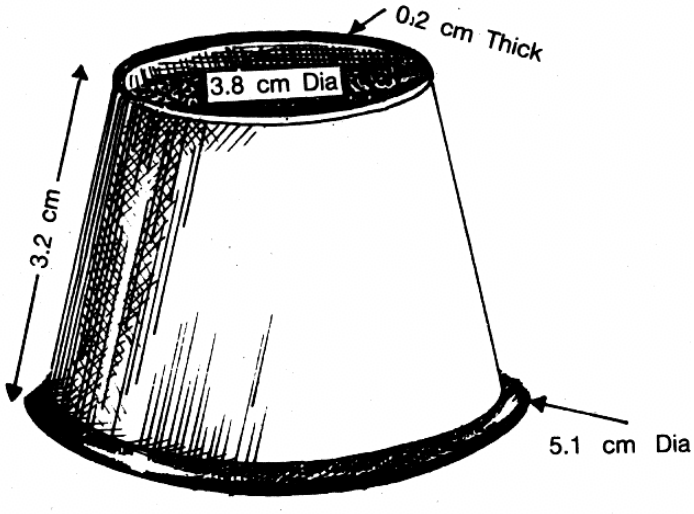
Grainage tray is made up of wooden/metal frame with wire mesh bottom. It is used for preservation of moths as it allows draining off the last excreta / urine passed by the moths before and after copulation.

9. Table and Stool



These table and stools are made up of either wooden or steel. The dimensions are given with the picture however it may be altered depending on the requirement. These are used for preliminary examinations of the cocoons, microscopic examination of moths during pebrine inspection and also for egg processing.

10. Cellule



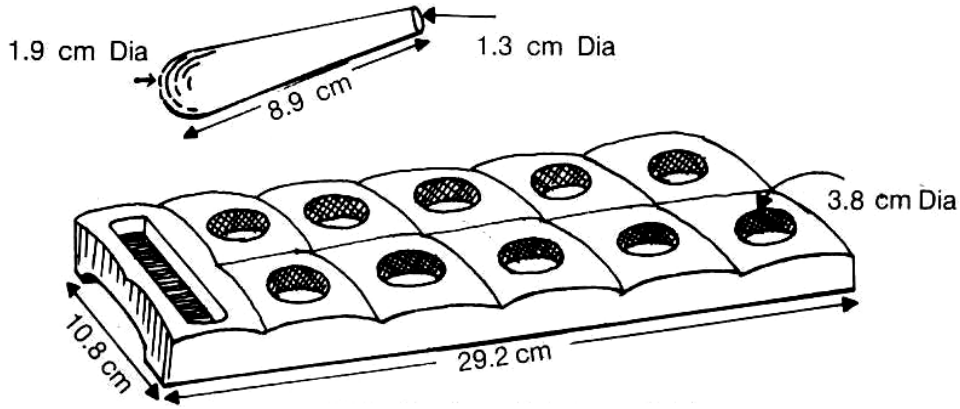
This is one of the important equipments used in the grainages. It is used during pairing of male and female moths; and oviposition as it protects the moths from other unpaired / stay male moths. Also, it avoids mother moths moving from one place to

another place and ensures uniform egg laying in one particular area. It is made up of PVC and black or blue in colour.

11. Mortar and Pestle

Mortar and pestle is made of porcelain and used to crush the mother moths for microscopic examination specifically sample/mass mother moth testing.

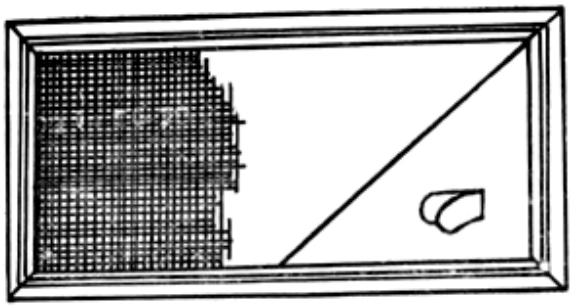
12. Moth Crushing set



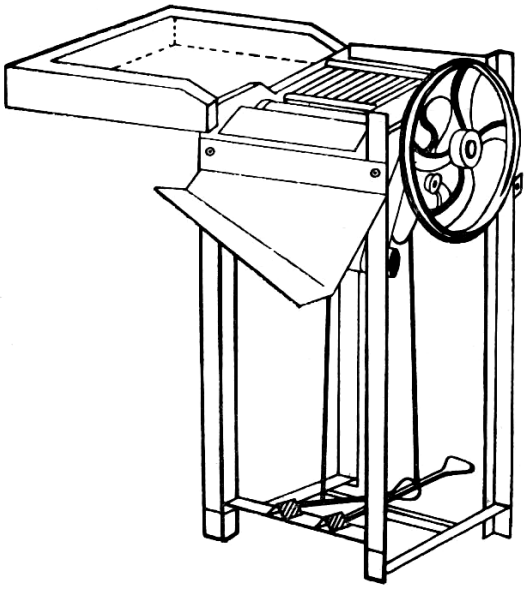
Moth crushing is also made of porcelain and is used to crush the mother moths for microscopic examination specifically for individual moth testing during the preparation of reproductive seeds at P_3 and P_2 stations of silkworm seed organization

13. Loose Egg Box

Generally silkworm seeds are available in two forms *i.e.*, layings and loose eggs. For the preparation of layings, female moths are allowed on the brown paper for laying the eggs. But in case of loose egg preparation, the eggs laid on starch coated brown paper are washed, weighed and filled in the box made up of wooden frame with muslin cloth.



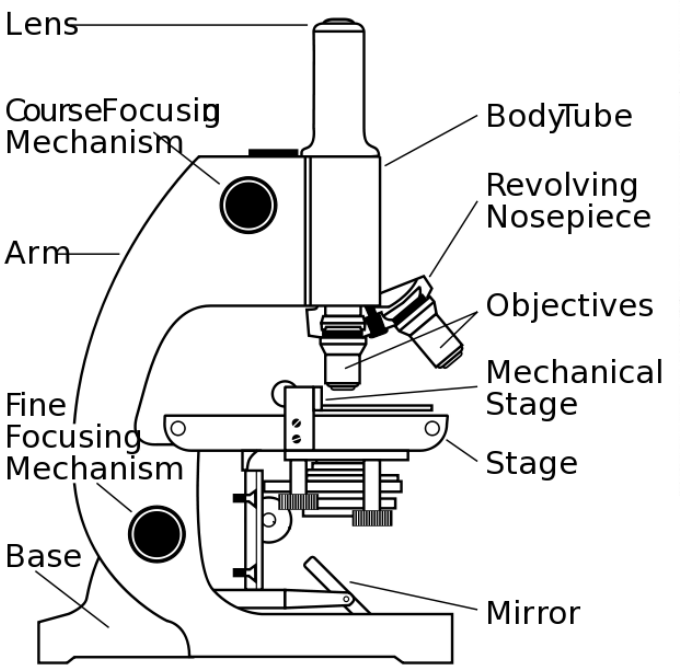
14. Cocoon Deflossing Machine



Cocoon deflossing machine is used to defloss the seed cocoons in grainages. During preparation of hybrids, both sexes of required component breeds should be preserved separately to avoid selfing or inbreeding. For this purpose cocoons should be cut open and collect the pupae for preservation. Therefore before cocoon cutting bivoltine cocoons should be deflossed.

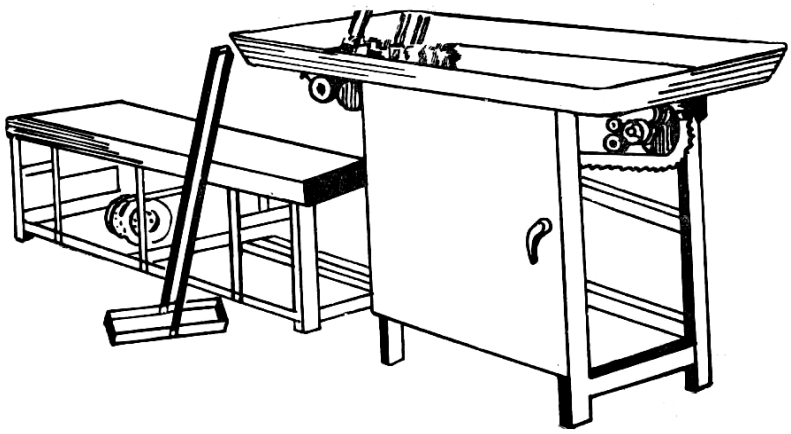
15. Microscope

Light microscope / compound microscope is used in pebrine test with 40-45 x objective lens and 10-15 x eye piece lens. However, 600 x magnification is ideal for pebrine detection.



16. Cocoon Cutting machine

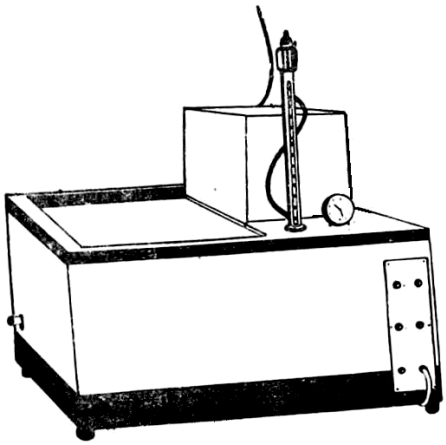
After deflossing, the bivoltine / hard cocoons are subjected to cutting for collection of pupae and sex separation. Otherwise the bivoltine moth emergence percentage will be reduced due to hard shell.



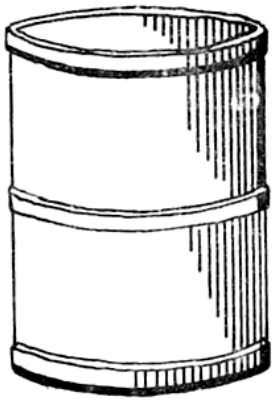
17. Refrigerator

Refrigerator is used to preserve small amount of cocoons for the purpose of synchronization of moth emergence. In addition it is used to preserve female/male moths before copulation for purpose of synchronization. Also, male moths after first mating and before second mating may be preserved.

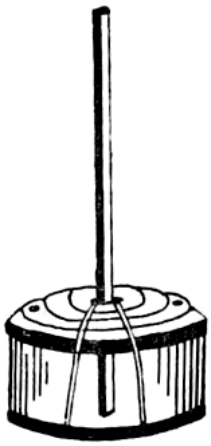
18. Acid Treatment bath, Acid Tub, Thermometer and Hydrometer



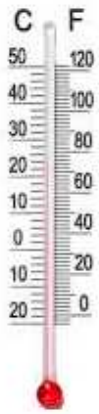
Acid Treatment Bath



Acid Tub



Loose Egg Container



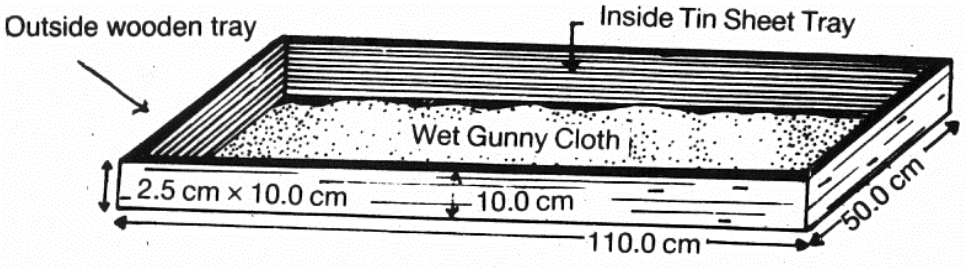
Thermometer

Hydrometer

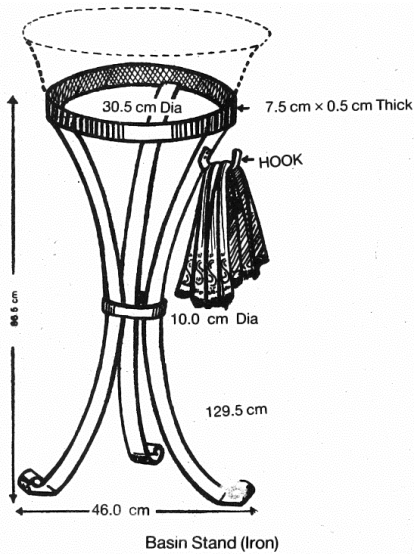
Acid Treatment bath is used for acid treatment of bivoltine eggs. Acid Tub is used to hold the hydrochloric acid during acid treatment and it is made up of non reactive material to HCl. Thermometer is used to set the temperature of acid, the required temperature is 46.1 C. Hydrometer is used to set the specific gravity of the acid and the required specific gravity of the acid is 1.075 for 4-5 minutes.

19. Foot Cleaning Tray

This is made of metal containing gunny cloth moistened with disinfectant for disinfection of feet while entering tha graunage.



20. Basin Stand



Basin Stand (Iron)

These are made of metal and are intended to hold a basin containing formalin or any other disinfectant. This is used for disinfection of hands while entering the grainage.

In addition, other equipments like craft paper to preserve the pupae for emergence, working stands to keep the trays at working height, egg cabinet for keeping the eggs, hot air oven to dry the moths, zinc trays, washing equipments such as trays, basins, room heaters to raise the temperature, humidifiers for maintaining the humidity, air conditioners *etc*.

Experiment No. 4: Seed Cocoon Processing.

Procurement of seed cocoons

The basic criteria for procurement of seed cocoons are, that it should be pebrine free and confirm to the norms, especially regarding the pupation rate and other norms fixed according to the season. In the cocoon market, the grainage authorities will purchase the required quantity of multivoltine cocoons in the regulated cocoon market as per the existing law. Generally half quantity of bivoltine cocoons should also purchased from bivoltine cocoon markets for the preparation of hybrid seeds. After purchase, the seed cocoons are packed loosely in perforated boxes or bamboo baskets in small quantities and are transported during cooler hours of the day to minimize the pupal mortality. If the live cocoons are tightly packed, they get heated up due to respiration. Exposure to hot sun results in dead cocoons and poor emergence.

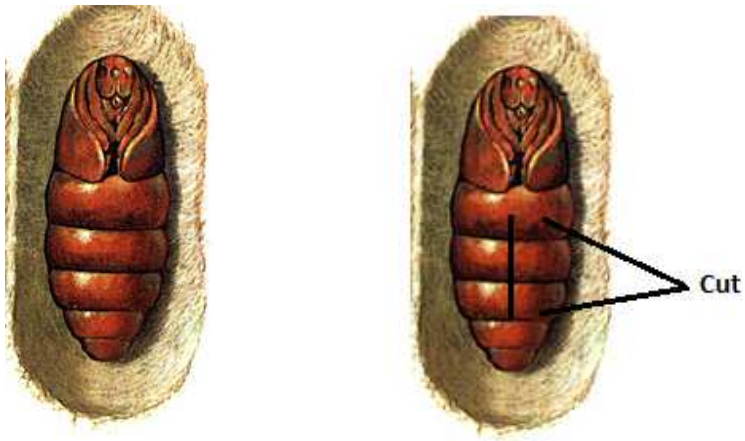
Preliminary Examination/Selection and sorting of cocoons

In order to produce good quality and healthy eggs the seed cocoons used for the purpose must be of high quality and in good health and therefore, the seed cocoons after arriving at the grainages are subjected to rigid selection. In selection only sound and uniform cocoons conforming to the characteristics of the race are selected and defective and deformed cocoons, under sized and over sized cocoons, double, stained and dead cocoons, uzi infested *etc.*, are rejected.

Advance detection of pebrine disease, if any before the commencement of operation of each batch helps in averting great loss to the grainages. This is facilitated by investigations at three stages *i.e.*, Pupal test, Forced eclosion test and First day moth examination. Of the three tests, pupal test is ideal as it gives ample time to send the cocoons for reeling unit if they are infected by pebrine.

Pupa Test

In this test, the gut of pupa is more reliable for pebrine detection than the entire pupa. For this purpose, the pupa is cut ventrally just below the wing bud by a scissor by holding the pupa between thumb and for finger in left hand. After cutting the pupa is pressed gently. The mid gut oozes out as a brown body from the cut portion. This mid gut is collected and crushed with few drops of potassium hydroxide in a moth crushing set. The fluid is taken on the slide and examine under the microscope with 600 X magnification.



Pupa before cut **Pupa after cut**

If the stock is suffering from pebrine, the entire batch of cocoon is rejected and sent to market. Such cocoons should never be used for preparation of silkworm seed under any circumstances.

Preservation of seed cocoons

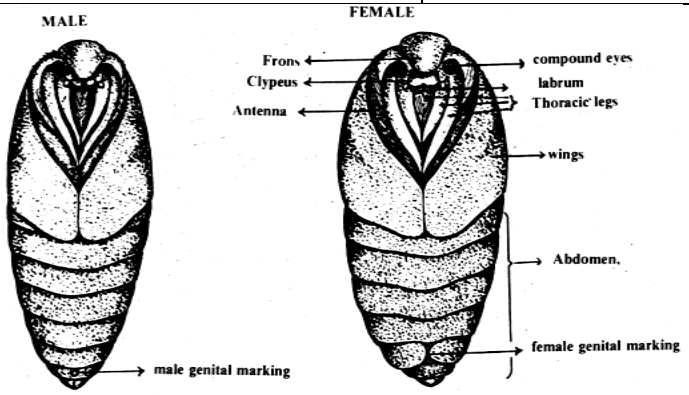
The cocoons are preserved in single layer in well ventilated rooms under natural light and dark conditions. Exhaust fans in cocoon preservation and moth emergence rooms are essential to expel foul gases and dust. Different component races are to be preserved in separate rooms.

Sex separation at Pupal and Moth stages

In the case of preparation of industrial hybrid seeds, the sexes must be separated before selfing with in the same parental race. This sexing may be carried out either in the larval or pupal or moth stage.

In case of pupal stage sexing, the cocoons are deflossed and cut at one end to remove the pupa for determination of sex. Cocoon cutting improves eclosion percentage in case of bivoltine cocoon, where the shell is hard and compact. Male and female pupae are separated in the pupal stage, based on the following differences.

Sl. No.	Male	Female
1	Smaller in size	Large in size
2	Narrow pointed abdomen	Broad abdomen
3	Small dot like mark on the ventral side near the top demarcation line of 9th abdominal segment	'X' mark seen on the ventral side of 8th abdominal segment



In case moth stage sexing, the main differences between the sexes are given below

Sl. No.	Male	Female
1	More active	Less active
2	Smaller in size	Large in size
3	Bigger antennae	Smaller antennae
4	The caudal end has a pair of hooks known as harpes helping in copulation.	The caudal end has a median knob like projection with sensory hairs, which is protruded and retracted to expel the pheromones (Ovipositor)

Female and Male Moths

Knob like ovipositor *1-Claspers; 2- Aedeagus*

Abdominal end of Female and Male Moths

Synchronization of Moth Emergence

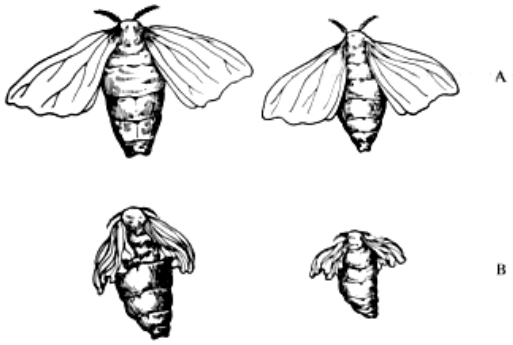
Moths of the component races are made to emerge on the same day, so that male and female moths are readily available for hybridization. This is referred to as

synchronization. Planning for synchronization starts at the time of brushing of the parental breeds. However, there may be some differences in spinning due to rearing conditions of the farmers. In such cases, emergence of moths in the two strains may adjusted by selecting cocoons of matching spinning date. In case synchronizing batches are not available, emergence of earlier bathes can be delayed by refrigerating the cocoons. The cocoons or pupae should be refrigerated preferably on the 7^{th} or 8^{th} day after spinning *i.e.*, 4-6 days after pupation, at 5-10°C. Such refrigeration should be limited to 3 days for female and 7 days for males.

The emerged male moths may be refrigerated at 5°C up to 7 days. The refrigeration of female either in the pupal or moth stage should be done as a last resort. Moreover refrigeration should be restricted to any one stage, either in the pupal or moth stage.

Experiment No. 5: Moth Emergence.

Selection of Moths



A – Health Moths; B – Unhealthy Moths

Different breeds of silk moths emerge at different intervals and it is about 12-14 days and also it is depending on the environmental temperature. The cocoon/pupa preservation rooms should be kept dark a day before emergence and bright light is switched on in the early morning by about 5 or 6 am., on the day of emergence. Each days' emergence will be over by about 8 am and it may extends over 3-4 days.

As soon as the moths emerged they start to get pair. For preparation of hybrids male and female moths should be picked as early as possible. After collection healthy, robust moths (as shown in the above picture) selected for hybrid preparation.

Coupling and Decoupling

The female moths are spread in a tray and the male moths of the desirable hybrid component are evenly distributed over the females. Generally excess males are distributed to facilitate quick mating. In about 15 minutes, the male and female moths pair. The excess males are collected for cold storage. Coupled moths are undistributed for 3 hours under normal day light condition. After the required period of coupling, the pairs are separated by holding the female moth and gently sliding the male. This facilitates easy separation without injury to the female reproductive organs.

Female and Male moths

Paired Moths

Oviposition and Preparation of Disease free layings

This is nothing but the process of egg laying by the female moth.

When eggs are required to be laid on cards, the mated females are placed on the egg sheets and each moth is enclosed in a cellule. The cellule isolates the eggs laid by each moth; facilitate individual moth examination and elimination of eggs laid by diseased moth.

In case of preparation of loose eggs, a unit number of female moths are allowed to lay eggs on starched paper or cloth, with in a wooden or plastic frame. The number of moths vary from 30-200 according to the health of the batch and convenience.

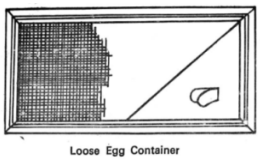
Moths are allowed to lay eggs for 24 hours in a dark room with 25°C and 75-80% RH. Then mother moths are examined for the pebrine disease and eggs free from pebrine disease are qualified for rearing.

Preservation of Male Moths

Female Laying Eggs

Layings

Loose Eggs Preparation



Loose Egg Box

Surface disinfection of eggs

After mother moth examination, the egg sheets or loose eggs in a container are dipped in 2% formalin solution.

1. This helps in eliminating surface contamination.
2. Formalin increases the adhesive capacity of eggs to the egg sheets.

Subsequently, the sheets are washed and dried under shade and preserved under optimum temperature (25°C) and RH of 80%.

Preservation and Handling of hibernated Eggs

In univoltine and bivoltine races, the eggs enter into diapauses in 40-50 hours after oviposition at 25 °C. Eggs when laid are yellow in color. Gradually they turn

brown and then purplish brown. Such eggs do not hatch unless they are activated by cold temperature. This is naturally achieved in temperate areas. In tropical countries, the winter condition could be stimulated by preserving the eggs at required temperature in a cold storage. Such cold storage of eggs is carried out by following specific schedules. Low temperature preservation terminates the diapauses and activates the eggs to hatch.

The schedule comprises 3 distinct phases *viz*., storing at 25 °C, cold storage at 5°C and intermediate phase, where the temperature is gradually lowered from 25°C to 5°C.

Short term refrigeration for 3 months:

The eggs are kept at 25°C for 3 days and then cold storage for 90 days at 5°C. The cold storage and release of eggs should be through an intermediate temperature of 15°C for 6-12 hours.

Refrigeration for 4 and 6 months:

The preservation period at different temperature is given below

Hibernation Period	Temperature							
	25°C	20°C	15°C	10°C	5°C	2.5°C	5°C	15°C
4 Months	10 days	2 days	2 days	3 days	50 days	50 days	-	1 day
6 Months	20 days	15 days	10 days	10 days	50 days	60 days	5 days	2-3 days

The eggs stored according to the above table can be released 15 days earlier or later than the scheduled period, without affecting the hatching. After releasing the eggs from 2.5°C, the eggs must be kept at an intermediate temperature of 15°C for one day and then transferred to incubation room.

Long Term Refrigeration (10 Months):

The schedule for preserving the eggs for 10 months is given in the following table

Hibernation Period		Temperature								
	25°C	23°C	20°C	15°C	10°C	5°C	2.5 C	15°C	2.5°C	15°C
10 Months	40 days	20 days	30 days	25 days	25 days	60 days	50 days	3days	30 day	2-3 days

Experiment No. 6: Mother Moth Examination.

After the female moths have laid their eggs they are subjected to microscopic examination to see if the moth is free from disease (pebrine) or not. Since pebrine spores are easily transmitted from diseased mother moths to the eggs, examination of the female mother moths must be carried out to ensure that their layings are free from disease. Moth examination is of 3 types.

They are

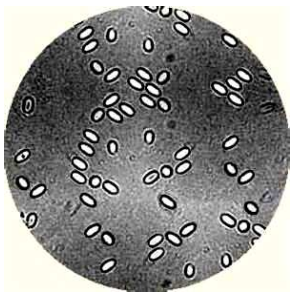
1. Individual Mother Moth Examination
2. Sample Moth Testing
3. Mass Moth Examination.

Individual Mother Moth Examination

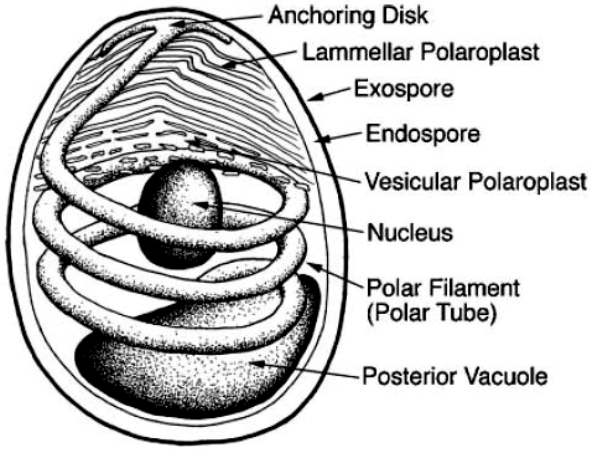
Individual moth examination is ideal to check the disease but laborious. Thergore, this method is done during the preparation of reproductive seeds at seed multiplication centers. Individual moth is taken and crushed in a moth crushing set with 0.25 ml of 2% potassium hydroxide solution. A drop of crushed fluid is taken on a glass slide and a cover glass is placed over the drop of fluid. Then, this is observed under a microscope with 450 - 600 x magnification. If the sample contains pebrine spore then such lot should not used for seed production.

In case of sample mother moth testing, only 20% of the emerged moths picked at random. In this method instead of crushing individual moths, two moths are crushed together.

The mass mother moth examination is quick and dependable method for pebrine disease and can be practiced in commercial grainages. In this case sample moths are drawn considering the number of moths in to a lot to be examined. Each sample comprises 20-30 moths.



Pebrine Spore Under Microscope



Enlarged Spore

Experiment No.7: Acid Treatment of Bivoltine Eggs.

Multivoltine breeds never undergo diapauses, but univoltine and bivoltines strains undergo diapauses or hibernation. Therefore to check the diapauses and to rear the worms in case of univoltine and bivoltine races, artificial hatching is done by two methods.

1. **Cold Treatment/ Physical methods:** In this method eggs are allowed to complete the hibernation period under cold conditions.

2. **Chemical Method / Acid Treatment:** *i.e.*, cold or hot acid treatment
   Of these two methods, hot acid treatment is more popular.

Hot Acid Treatment:

This treatment is practiced just between 15-20 hrs of oviposition *i.e.* before the egg show diapausing characteristics should be treated. When the treatment is to be postponed the eggs are preserved at 5°C at 20th hour. The period of cold storage should be within 5 days. During this period eggs can be taken at any time for acid treatment, but before the treatment eggs are exposed to 25-26°C for 2-3 hours. Hydrochloric acid of specific gravity 1.075 (15%) is heated to a temperature of 46.1° and the silkworm eggs are immersed in the acid for 5-6 min. The specific gravity is as measured at 15°C. Then wash the eggs in running water to remove traces of acid and dry under shade. "Now the eggs are ready for Incubation"

Experiment No. 8: Identification of different types of eggs.

Aim: To identify different types of eggs.

Introduction: Silkworm *Bombyx mori* lays different types of eggs like hibernating and non hibernating eggs and also, depending on voltinism and seasons. In addition, within the same strain /breed different type of eggs are observed depending on stages of embryo developing inside. Therefore, this experiment gives clear idea of embryonic stages, facilitates handling of eggs.

Requirement: Different types of eggs.

1. **Non hibernating eggs:** Multivoltine pure breeds or Multivoltine x Multivoltine breeds lays only non diapausing eggs. These non hibernating eggs are light yellowish in colour in the beginning, but later turn light pinkish indicating the embryonic development. The first generation bivoltine eggs are non hibernating eggs, but the colour changes to purplish brown after 24 h of laying.

2. **Hibernating Eggs:** The univoltine breeds lays only hibernating eggs. The bivoltine breeds lays both non hibernating and hibernating eggs *i.e.*, first generation eggs are non hibernating and second generation eggs are hibernating. The egg colour is yellowish in the beginning, but later turns to purplish brown after 24 h of laying indicating the development of Ommachrome pigment.

3. **Eye Spot Stage:** In both hibernating and non hibernating eggs, on 8th day of embryonic development shows eye spot on the surface of the chorion. This is due to development of black pigment at mouth and head region of the embryo and appears as an eye. Hence the name eye spot.

4. **Blue Egg Stage:** On 9th day of embryonic development, the development of black pigment continues to remaining parts of the body. So the whole embryo becomes black in colour. But due to thick semitransparent chorion it appears as blue in colour. Hence the name blue egg stage.

5. **Hatched Eggs:** It can be identified as white empty coloured shell with one side opening.

6. **Unfertilized eggs:** It can be identified yellowish in colour without any changes.

7. **Dead Eggs:** It can be identified as black, shrunken eggs indicate the death of the embryo during development.

Experiment No. 9: Calculation of Hatching Percentage.

Aim: To determine the hatching per cent age in the given laying.

Introduction: Hatching of silkworm eggs starts early in the morning. In order to obtain uniform and maximum hatching, the egg cards at pin head or blue egg stage are kept at black box for one or two days before hatching *i.e.*, Black Box Technique. In this way the completely developed matured embryos are prevented from hatching and under developed immature embryos are given time to make up the development. Next day they are exposed to bright light. So that the larvae starts to hatch uniformly in response to photo stimulation given by the light. By this method hatching can be achieved 90-95% in one day.

Requirements: Hatched egg cards and colour sketch pen.

Procedure: Take a hatched egg card / laying and count hatched eggs, dead eggs and unfertilized eggs based on their colour separately using different colored sketch pens. Record the individual numbers.

Observation and Calculations:

Number of hatched eggs - ____ (A)

Number of dead eggs – ____ (B)

Number of unfertilized eggs - _____ (C)

Total number of eggs - _____ (D)

$$\text{Hatching per cent age} = \frac{\text{Number of Hatched Eggs X 100}}{\text{Total Number of Eggs}} = \frac{\text{A X 100}}{\text{D}} = \text{___ \%}$$

$$\text{Dead Egg per cent age} = \frac{\text{Number of Dead Eggs X 100}}{\text{Total Number of Eggs}} = \frac{\text{B X 100}}{\text{D}} = \text{___ \%}$$

$$\text{Unfertilized Egg per cent age} = \frac{\text{Number of Unfertilized Eggs X 100}}{\text{Total Number of Eggs}} = \frac{\text{C X 100}}{\text{D}} = \text{__ \%}$$

Report: The hatching per cent age of the given egg card is _____ %.

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i. Sericulture Manual 2; Silkworm Rearing, FAO, Rome 1987.

ii. Manual on Silkworm Egg Production, CSB, India, 1988.

iii. Industrial Bivoltine Grainage for Tropics, CSR & TI, Mysore, 1986.