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Importance of Egg Incubation in Silkworm Seed Production

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The silkworm, *Bombyx mori* L., has undergone domestication for more than 3000 years, which has resulted in the gradual loss of its natural resistance to environmental stress. As a result, the insect has become highly sensitive to environmental conditions at every stage of its life cycle. Exposure to unfavourable environmental factors during the egg stage can lead to various embryonic abnormalities, ultimately reducing egg quality and causing embryo mortality. The severity of such damage largely depends on the extent to which environmental conditions deviate from the optimum levels. Therefore, maintaining suitable environmental conditions during this stage is essential to ensure uniform embryonic growth, proper development, and higher hatching percentage. Incubation refers to the process of maintaining silkworm eggs under optimal temperature, humidity, and photoperiod conditions to facilitate proper embryonic development and ensure the simultaneous hatching of healthy larvae. It represents the final stage of silkworm seed production and the first stage of silkworm rearing.

Objectives of Incubation

- To ensure uniform embryonic development and synchronized hatching of larvae on a single day.
- To regulate the hatching of different races on predetermined dates, thereby facilitating F1 hybrid seed production in commercial grainages.
- To maintain the vigour and health of newly hatched larvae.
- To preserve the voltinism characteristics of a particular race.

Activities Involved in Incubation

Disinfection of the Incubation Room

Disinfection is an essential step prior to the initiation of incubation. This process helps eliminate disease-causing microorganisms present in the incubation environment. Therefore, before starting incubation, the incubation chamber, equipment, and appliances must be thoroughly disinfected. In addition, surface sterilization of silkworm eggs is necessary to prevent contamination and ensure a disease-free incubation process.

Surface Sterilization of Silkworm Seeds

Surface sterilization is an essential step before transferring silkworm seeds to the incubation chamber. The seeds should be treated with 2% formalin solution for about 10 minutes to eliminate disease-causing pathogens present on the surface. Formalin acts as an effective disinfectant and destroys microorganisms that may have contaminated the eggs or seed sheets during the oviposition process. Silkworm eggs produced in loose form can also be easily sterilized by immersing them in 2% formalin solution for 10–15 minutes. In the case of sheet eggs, it is important to use good-quality egg sheets that can withstand immersion in formalin for the required duration without damage.

If surface sterilization is not carried out properly, pathogens present on the chorion (outer shell of the egg) may be ingested by larvae during hatching. This can lead to infection, causing larval mortality and creating a source of secondary contamination that may spread diseases to other larvae in the rearing batch. Therefore, improper surface sterilization can be a major factor contributing to cocoon crop losses. Silkworm eggs can be safely surface sterilized at any stage of embryonic development, except during the pin-head stage and blue-egg stage, as treatment during these stages may affect embryo viability. In addition to the initial sterilization carried out at the grainage, the process may be repeated at Chawki Rearing Centres (CRCs) as a precautionary measure if the seeds are transported before reaching the pin-head (head pigmentation) stage. This additional sterilization ensures that any contamination occurring during transportation is effectively removed. For loose eggs, the seeds should be taken out from the container, placed in a cloth or nylon bag, and immersed in 2% formalin solution for about 10 minutes. After treatment, the eggs are removed, dried properly, and then processed for further incubation procedures.

Transportation of Silkworm Eggs

- Transportation of silkworm eggs is usually carried out during the incubation period; therefore, special care must be taken to prevent damage to the developing embryo.
- Eggs should be transported only during the cooler hours of the day, preferably early morning or late evening.
- Transportation should be avoided between the 4th and 5th day of embryonic development, as the embryos are highly sensitive during this period.
- During transport, eggs must be maintained under suitable temperature, humidity, and proper aeration conditions to ensure normal development.
- Eggs should not be transported in polythene covers or airtight containers, since poor ventilation increases the chances of egg mortality.
- Proper handling during transportation is essential to maintain egg viability and ensure healthy larval hatching.

Embryo Test

During the incubation period, embryo testing is carried out to monitor the progress of embryonic development.

This test helps in:

- Predicting the expected date of hatching.
- Confirming the proper growth and development of the embryo.
- Regular embryo testing enables better management of incubation and ensures synchronized hatching.
- Environmental Conditions for Incubation

Successful incubation depends on several environmental factors such as temperature, relative humidity, photoperiod, and aeration.

Temperature

Temperature plays a crucial role in determining the rate of embryonic growth and development. The optimum temperature required for silkworm egg incubation is $25 \pm 1^\circ\text{C}$. Maintaining this temperature also helps preserve the diapause characteristics of bivoltine races.

Humidity

Relative humidity is important for maintaining the moisture content of the eggs. Humidity 80% ensures uniform embryonic development and prevents desiccation of eggs.

Aeration

Adequate aeration is essential during incubation because silkworm eggs remain physiologically active throughout the developmental period. Proper ventilation supplies sufficient oxygen for the developing embryo and removes harmful gases, thereby supporting normal embryonic growth.

Photoperiod

Photoperiod plays an important role during the incubation of silkworm eggs. A photoperiod of 16 hours light and 8 hours darkness (16L:8D) up to the pin-head stage is essential for proper and uniform embryonic growth. Maintaining this light–dark cycle also helps in preserving the voltinism characteristics of the silkworm race.

Black Boxing

- Black boxing is a technique used to obtain synchronized hatching of silkworm eggs. In this method, the eggs are kept under complete darkness for about 48 hours prior to hatching, when they have reached the head pigmentation stage.
- The ideal time to begin black boxing is when more than 50% of the eggs reach the head pigmentation stage. For CSR bivoltine breeds, black boxing should be carried out about 60 hours before hatching in the case of acid-treated eggs, and around 72 hours before hatching for hibernated eggs.
- This process is usually done by covering the eggs with black cloth or black paper, or by making the incubation room completely dark. On the day of hatching, the eggs are exposed to diffused light, which stimulates the larvae to hatch simultaneously.

Brushing

- Brushing is the process of transferring newly hatched larvae from the egg sheets to the rearing bed and providing them with mulberry leaves for feeding.
- On the expected day of hatching, usually around 6:00 a.m., the eggs are exposed to light to initiate hatching. Natural daylight is generally sufficient to stimulate this process.
- Immediately after hatching, the larvae should be brushed gently onto the rearing bed and provided with tender chawki mulberry leaves containing about 75% or more moisture, which are suitable for feeding young larvae.

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