# CHAPTER - II

# REVIEW OF LITERATURE

**2.1. Environmental effect on muga silkworm seed production:**

Muga-the golden yellow silk, is produced by the insect species *Antheraea assama* Westwood. (Lepidoptera: Saturniidae). It is multivoltine and polyphagous in nature. It feeds on various plants, viz *Persea bombycina* Kosterm (*Machilus bombycina* King), *Litsea monopetala* Pers, *Litsea polyantha* Juss, *L. citrata*, *L. Salicifolia*, *Magnolia sphenocarpa* and *Zizyphus jujuba.* Rearing of muga is conducted on *Persea bombycina* and *Litsea polyantha* in North-Eastern region of India, mainly in Assam and West Bengal particularly in CoochBehar district.

Muga, which is produced nowhere else in the world except in Assam, has of late been introduced in Mizoram, Nagaland and Arunachal Pradesh for production of seeds at higher altitude (Yadav and Goswami, 1987). Then muga finds its way to West Bengal after establishment of RMRS Research extension centre at CoochBehar. After conducting several trial rearings in different villages of Cooch Behar it has been established that muga culture is quite promising in this new zone (Singha *et al.,*1991). Different opinions are that the species is not only confined to north-eastern region rather it has a wide distribution both within the country and abroad. In India, the species is also available in Himachal Praesh, Uttar Pradesh, Sikkim, Gujarat and Pondicharry . It is also available in Bangaladesh, Srilanka and Indonesia. (Sengupta and Srivastava, 1995).

However, traditional practices in muga culture are mainly confined in Assam and North-Eastern States. The muga culture has become a part of the Assamese culture. Probably due to its restricted habitat in the North-Eastern Region, the muga silk insect, *Antheraea assama* has not drawn much attention of the people from the other parts of the world. (Thangavalu *et al* 1988).

Introduction of muga culture outside the North-Eastern India have not met successfully, probably due to lack of suitable climatic conditions. The climate of the North- Eastern India ($21^0$N to $29^0$30'N latitude and $89^0$46'E longitude) is very distinctive, which is sub-tropical having four distinct seasons viz. Summer (May-September), autumn ( October-November ), winter (December – February ) and spring (March – April). The summer is hot and humid with very high rainfall. The maximum temperature never rises above $34^0$C at the sea level and the high temperature is recorded only for a few days. Autumn and Spring are very pleasant and the minimum and maximum temperature ranges form 16$^0$C to 20$^0$C and 20$^0$C to 25$^0$C during these two season. Winter in moderate and the minimum temperature does not fall below 7$^0$C at sea level. Perhaps the distribution of *Antheraea assama* is restricted to North Eastern India due to this unique climatic condition.

Geographically Cooch Behar district of West Bengal 25$^0$57' and 27$^0$ N latitude and 88$^0$25' and 89$^0$54' E longitude can be considered as a contiguous of Assam having more or less similarity in agro-ecological, socio-ethno-economic background. Climate of Cooch Behar district is also subtropical, humid with high rainfall having short spell of winter. Maximum rainfall receives during June-September and maximum temperature never rises above 33.2$^0$C with an exceptionally high 2-3 days peak of 35-40$^0$C. Autumn and Spring are very pleasant and the temperature during this period ranges from 17.75$^0$C to 25.11$^0$C. Winter is moderate and the minimum temperature does not fall below 6$^0$C. Therefore, climate conditions of the district is more or less similar to that of Assam at least those places where muga culture is in vogue. This agro-climatic situation stimulates muga culture in Cooch Behar district of West Bengal (Ray, 2003).

Muga culture in Uttar Pradesh has also been tested by Sengupta and Srivastava (1995). The climate of the region varies according to their elevation from hot in the foothills to the freezing point in the Himala yas. The hills of Uttar Pradesh can be classified into the higher hills simulating temperate conditions and the middles hills being sub-tropical in nature. Doon valley particularly has been explored (77$^0$35' and 78$^0$2' E and 29$^0$57' and 31$^0$2' N). The climate of the valley is temperate. Annual atmospheric temperature varies between 4 ± 1$^0$C to 41 ± 10C. Monsoon season extends from mid June to early October and annual precipitation varies between 1600 mm and 2500 mm, highest rainfall usually recorded during August. Climatically, three well-defined seasons are experienced in the valley. Summer from March/April to mid June – temperature ranges between 15$^0$C t o 35$^0$C with a brief spell of high temperature reaching up to 41$^0$C. Monsoon begins from mid June and lasts upto early October. Average temperature ranges from 20$^0$C to 32$^0$C while relative humidity sometimes reaches up to 95% and rainfall is generally maximum during August. Winter season begins from November and extends upto February, when the average maximum temperature is about 21$^0$C. In winter, about 50 mm to 60 mm rainfall is recorded. Therefore, the climate of Dehradun is ideal for muga culture.

Research works are mainly confined to North-East India. Among the seven North-Eastern states, muga production is confined mainly to the state of Assam. Assam is the only state for production of reeling cocoons, whereas other states have the privilege of producing major quantity of seed cocoons for commercial multiplication in Assam. Problems of muga industry includes vagaries of weather, pests, diseases, predators, untimely seed crop rearing, aged and improperly maintained plantation etc. Added to these, there is no specific race or seed organization for regular multiplication of basic seed meant for the production of commercial seed. Muga Seed Development Project was launched in the '80s to overcome the basic seed shortage but it could not fulfill the complete demand of basic seed. More then 95% of the commercial seed production is being vested with private rearers without any proper mother moth test. Hence as long as seed brood is free from pebrine disease, the seed produced by farmers may help them to realize good corps provided leaf quality and weather conditions are favourable. Muga silkworms thrive well when temperature and humidity ranges between 24-30$^0$C and 85-90 % RH respectively. It is difficult to posses the optimum condition all through the period of rearing especially during seed crop rearing.

Timely supply of adequate quantity silkworm seeds can only sustain the industry by ensuring successful cocoon corps. (Francis *et al.*,1995). The problem is that the growth and development of muga silkworm is very much susceptible to the climatic conditions and it reacts with every change in the environment, which reflects on the commercial characters of the silkworm. Being multivoltine in nature, it has five to six generations in a year. The climatic conditions favours the rearing of the muga silkworm during autumn (Kotia) and spring (Jethua) and hence commercial rearing is restricted to these two seasons only. However, as it is poly voltine in habits, rearing of unfavorable summer (aheruā and bhodia) and winter (Jarua) are also unavoidable and quite essential for survival of the silkworm. The muga seeds are multiplied during these unfavorable seasons only and hence known as seed broods. The productions during the seed broods are very poor, unsatisfactory and unpredictable. Occasional hailstorm, frequent raining and high temperature during summer and severe cold and poor growth of food plants during winter are inimical to the muga silkworm. (Sengupta *et al.,* 1992).

Being reared outdoor, the larvae of *A. assama* like those of *A. yamamai, A. pernyi* and *A. mylitta* have adjusted themselves to a certain degree against all kinds of selection forces of Nature (Crotch, 1956; Yamazaki, 1959). Various factors have shown to affect the success of silkworm cocoon production including environment (37%), leaf quality (38.2%), rearing technique (9.3%), silkworm race (4.2%), silkworm egg quality (3.1%) and others (8.2%). (Matsumara,1975). In 1933, Kogure opined that the silkworm could tolerate temperature upto $30^0$C. Silkworm larvae become weak by rearing at higher temperature than $30^0$C (Upadhyaya and Mishra (1991) and hampered growth enhancing the mortality (Mathur *et al.*, 2003). While Nirmal Kumar *et al.* (2005) observed on bivoltine *Bombyx mori* that higher temperature induces the silkworm to develop faster, mature early and spin the cocoon quickly. The opposite happens when temperature falls below the normal range. It has been recorded that environmental conditions are the main cause of variability in *A. mylitta* as the fecundity, hatchability, cocoon weight, shell weight, absolute silk yield, filament length, denier and sericine percentage varied during different season (Srivastava *et al.*, 1998).

In *Samia ricini*, the commercial traits such as cocoon weight, shell weight, pupal weight varied significantly during different seasons. Lower days of rearing period and life cycle during summer was associated with lower value of cocoon weight and shell weight and reverse was the case in winter season (Kar and Guru, 1998).

*A. assama* in relation to temperature and relative humidity in all the six corps was studied by Sahu *et al.* (1978). Profound effect of temperature was observed in incubation period, hatching percentage, larval – pupal periods, coupling and oviposition. 23-$27^0$C and 70-77% RH appears optimum for coupling as well as oviposition, while 19-$26^0$C and RH of 70% above was found to the optimum for hatching.

In *Antherea assama,* there was steady and significant increase in the hatching percentage along with the rise in temperature from $17^0$C to$26^0$C, but a declining trend on hatchability was observed for $29^0$C to $35^0$C. The hatching percentage was significantly impaired at $35^0$C. On the other hand incubation period and hatching duration are inversely proportional to the rise of temperature (Barah *et al.*,1993). According to them, the humidity regimes under individual temperature treatment, the humidity regimes of 65%, 75% and 85% had relatively much less effect on the incubation period and hatching duration but showed a significant improvement on hatchability in between the treatments. But in all temperatures, the humidity above 90% showed a relatively impaired hatching, which was significantly inferior to the other humidity regimes. According to Wigglesworth (1972), 90% RH showed an advance effect on hatching, such as a result is possible when some hygroscopic substance in the egg chorion is affected. The high atmospheric temperature and low RH persisted for quite long time resulted in huge pupal mortality, only one peak of emergence of moths occurred as against usual two peaks, low fecundity and higher egg retention in ovary in tropical tasar silkworm, *A. mylitta*. (Mathur *et al.*, 2005). Studying on West Bengal condition Saha *et al.* (2007) also observed that hot and humid summer and high precipitation impede the rearing of production breeds.

Seasonal changing of photoperiod also have effect on seed crop rearing as the light and dark schedules of the natural day in animals induce different kinds of fluctuations in growth and development and a combination of physiological mechanisms (Denilevskii, 1965) and particularly in insect. Photoperiodism regulates the growth and various physiological mechanisms (Beck, 1980). Masaki (1972) observed that photoperiod has a positive effect on synchronization of adult emergence in cricket. In each insect in population rhythms, circadian events compose 'allowed zones' or 'gates' through which egg hatching, eclosion or release of hormones may occur (Saunder, 1982).

Grainage performance during the pre-seed and seed corps and especially during Chotua and Aherua seasons suffer due to low humidity and low temperature (Chotua) and high temperature ranging from 26-36$^0$C and high humidity (74-76%) during Aherua and Bhodia seasons resulting in requirement of more number of cocoons for producing one Dfl (cocoon : Dfl ratio 5:1 or even more ), poor moth quality and low to very low hatching (0-25%) due to exposure of the late age worms to high temperature (36$^0$C) during out door rearing and spinning under indoor condition. This has been identified as one of the major problems in muga culture that causes shortage in production of main commercial seed during kotia crop (Sahu, 2004).

In mulberry silkworm the effect of photoperiod on pupal characters and hatching has been reviewed (Janarthanan *et al.*, 1994; Benchamin *et al.*, 1990) and gating phenomenon has been demonstrated in egg hatching (Sivarami Reddy and Sasira Babu, 1990). In tasar silkworm, short days promote diapause development, while long days maintain diapause (Sakate *et al.,* 1982).

The role of photoperiod in the developmental processes and in the induction of pupal diapause in muga silkworm *A. assama* was studied. During short day length (LD 4.5 : 19.5 and LD 6 : 18), photoperiods of high intensity slowed down development rates but were not effective in inducing diapause in this insect. The effect of photoperiod on oviposition and hatching were also studied. Female laid more eggs under short-day than under long-day conditions and the short-day condition also showed higher hatchability (Mahanta and Goswami, 1986). When the larvae reared in different photoperiodic regimes (24L, 18L : 6D, 12L : 12 D and 6D : 18L) and in different season (spring, early summer, late summer, late autumn), temperature influences the effect of photophase in case of developmental period, food utilization and growth. High temperature and constant light as well as low temperature short photaphase are found deleterious. The sensitivity to photoperiod decrease towards instar when the larvae are more sensitive to temperature.

In mulberry silkworm, incubation of eggs under complete light or complete darkness leads to irregular hatching thereby reducing the hatching percentage (Chowdhury, 1984) and 12-18 hours light and 6-12 hours dark is effective photoperiodic regime for perfect and uniform hatching. 12-18 hours light/day is favourable for the embryonic development and uniform hatching (Meenal *et al.,* 1994).

Taking all these environmental factors under consideration egg production challenges in muga silkworm has been studied in North Eastern States. According to them, the success of commercial crops highly depends on the success of seed crop rearing when the environmental conditions are hazardous. During June-August, the environment is very hot and humid with a temperature range of 25-38 $^{0}$C and heavy rainfall. Heavy loss of seed cocoons occur during this period. Moreover, the seed cocoons produced exhibit emergence of deformed moths, coupling inefficiency and infertility of eggs that badly affects the availability and fertility of eggs for the ensuing commercial crop rearing. Another seed crop rearing (including pre-seed) season in November – March when temperature fluctuates between 10 and 28 $^{0}$C with drastic fall during night. Nearly 50% or more loss of seed cocoons occur in the grainage causing shortage of seed for subsequent commercial rearing.

In newly explored areas, the scenario is not as poor as Assam. In Doon Valley, fecundity of the DFLs was higher than average fecundity found under Assam conditions. Hatching percentage was also recorded much higher (81%) is comparison to that in Assam condition (55%). These are enough scope for extension of mugaculture in the hills of Uttar Pradesh especially in Dehradun because due to adverse climatic condition, the rearing of muga silkworm particularly the seed crops are very uncertain in northeast India (Sengupta and Srivastava, 1995).

However, another extension center is West Bengal particularly Cooch Behar district where the climatic contiguity with Assam explores successful mugaculture. Upon preliminary observation by Ray, 2003, it can be said that October –November is the main commercial crop-rearing season. Surprisingly during March-April the seed crop-rearing season, the fecundity observed 162.50 much higher than Assam.

After that, an investigation on muga silkworm rearing as well as grainage was undertaken during nine different seasons : Feb.-Mar., April –May, May-June, July-Aug., Aug.-Sep., Oct.-Nov., Nov.-Dec., Dec.-Feb. and Jan.-Mar. to identify the suitable season for commercial and seed crop rearing for successful introduction of muga culture in Terai Region of West Bengal, India, a newly explored area. Rearing and grainage parameters were better during Oct.-Nov. followed by April-May and minimum during July-Aug. (Ray *et al.*, 2005).

**2.2 Effect of host plant on Seed production:**

Cocoon crop of silkworm depends upon the developmental vigour of the silkworm breeds, which in turn, is further influenced by the nutrient availability from the leaves fed (Ito and Arai, 1965). Plants with higher protein contents were more readily accepted and supported growth better than varieties with lower protein content (Soo Hoo and Fraenkel, 1966). Zhang *et al.* (1991) also reported that, increase of protein content shortened the larval duration, increased larval weight and digestibility. Moreover, the weight of pupae, fecundity and weight of eggs increased with the rise of protein content. Hamano and Tsuchida (1989) noticed that the mortality of larvae was lowered with the increase of protein content in diet. Since, the principal constituent of Silk is protein; leaves with high protein content are more readily accepted by the worms and promote growth better than the varieties with low protein content (See Hoo and Fraenkal, 1966). Li and Samo (1984) reported that lower values of leaf moisture and protein content in the food recorded lower rates of larval growth, body weight and cocoon weight. Therefore, there exists difference between the host plants with respect to nutrient content.

In mulberry silkworm, Satyanarayan Raju *et al.* (1990) observed that the performance of Kosen was highly satisfactory, while Venugopala Pillai and Jolly (1985) observed that $MR_2$ variety was superior and closely followed by Kosen so far as rearing performance was concerned. However, Ghosh *et al.* (1993) reported that leaf quality as well as yield of the $S_1$ variety was better in Malda district of West Bengal than other varieties studied. Subba Rao *et al.* (1987) recommended $TR_4$, $TR_{10}$ and $S_1$ varieties of mulberry for higher leaf yield in Jalpaiguri district under terai region of West Bengal. According to Das *et al.* (1995) nutritive quality of $C_{763}$ was found superior to that of other varieties (Viz. $S_1$, $S_{779}$, $S_{1635}$, $C_{1730}$, $C_{763}$, $C_{776}$ and Kajli) followed by $S_{1630}$ and $S_{1730}$ in gang tic planes of West Bengal.

Nutrient content observed best in Kosen followed by $TR_{10}$ and rearing parameters observed best in $TR_{10}$ followed by Kosen. However leaf yield of $S_1$ is highest, nearly double than Kosen and $TR_{10}$ suggesting selection of $S_1$ for bivoltine silkworm rearing in Terai region of West Bengal with culture compensation (Ray *et al.*, 1998). Ghosh *et al.* (2007) reported that $C_{1730}$ is the best variety so far as rearing of silkworm is concerned followed by $S_{1630}$, $BC_259$, $TR_{10}$, Kosen, $S_1$, Bombai local and Kajli in Eastern India.

In non-mulberry section, the influence of host plant was not reported so vividly because of poor systematic plantation mainly depending on forest. Tasar silk includes tropical tasar and oak tasar. While tropical tasar is produced by the silkworm *Antheraea mylitta* which feeds primarily on *Terminalia anjuma, Terminalia tomentosa* and *Shorea robusta*, the oak tasar is produced by *Antheraea proylei, Antheraea pernyi* and *Antheraea roylei* fed on different species of *Quercus sp* mainly on *Quercus lencotricophora.*

Out of the total 76.1 million ha. of forest area available in the tropical tasar cultivating states (Jharkhand, Bihar, Orissa, Maharashtra, Chhattisgarh, Madhya Pradesh, Andhra Pradesh and West Bengal) nearly 19.05 million ha of tasar food plants are estimated to be available in the tropical belt of which *Shorea robusta* (Sal) accounts for 87%. The other two primary food plants viz. *Terminalia tomentosa* and *T. arjuna* and the secondary food plants (Zizyphus jujuba, *Terminalia chebula, T. belevica, T. catappa, Lagerstroemia paniculata, Anogeissns latifolia, Syzygium cumini* etc.) account for 1.4 million ha. (13.1%) (Mohan Rao, 2007).

The states where oak tasar culture is practiced are Assam, Manipur, Himachal Pradesh, Jammu and Kashmir and Uttaranchal. Different species of oak tasar food plantation are spread over different districts of the states. It is estimated that 195103 ha of *Quercus lencotricophora*, 72735 ha. of *Q .floribunda* and 26577 ha. of *Q. semicarpifolia* are available in Uttarcnchal, the leading state in oak tasar culture. However, *Q. incana* and *Q. semicarpifolia* are available in the North-West, while *Quercus dealbata* and *Q. serratta* in the North-East (Khatri *et al.,* 2007). Effect of different morpho types of *Gueraus semicarpifolia* on the rearing of *A. proylei* revealed that rearing on thick, non-spiny leaves of *Q. semicarpifolia* were found suitable for better yield on *A. proylei* (Raja Ram *et al.,* 1998).

Among the three natural host plant, *Terminalia tomentosa, Terminalia arjunna* and *Zizyphus jujuba. T. tomentosa* was found to be most suited host plant for the larva of *A .mylitta* in all respects (Rath, 1998).

Eri silkworm (*Samia ricini* Donavan) is a polyphagous insect and feeds on a wide range of host plants. At present, as many as 24 plant species are known to host eri silkworms. *Ricinus communis* Linn. (Caster) and *Heteropanax frograns* Seen. (Kesseru) are the primary hosts and are principally utilized for feeding eri silkworms. *Manihot utilissima* Pohl, (Tapioca), *Evodia fraxinifolia* Hook. (Payam), *Ailanthus grandis* Prain (Barpat) and *Jatropha curcas* Linn. (Bhotera) are the secondary host plants and are used less frequently. Plants like *Plumeria actifolia* Poir (Gulancha), *Gmelina arborea* Roxb. (Gramani), *Pyms pashia* Ham, (Patangi), *Xanthoxylum alatum* Roxb. (Jaunsar) and *Stercvlia villosa* Roxb. are the tertiary host plants and their leaves are rarely used for feeding during acute shortage of leaves of primary food plants for some specific and shorter periods of larval life (Bindroo and Khan 2007).

Som (*Persea bombycina* Kost.) and Soalu (*Litsea polyantha* Juss) are the two principal food plants and Mejankari (*Litsea cubeba*) and Dighloti (*L. salicifolia*) are the secondary host plant of muga silkworm. Som is more prevalent in upper Assam, whereas soalu is more common in lower Assam. The lower Assam rearers now prefer som trees for production of muga cocoons due to their long life span and more resistance to stem borer attack as compared to soalu trees. Depending upon the leaf shape and size, four varieties of some have been identified of which Naharpatia is better than Ampatia, Jampatia and Kathalpatia. Similarly, in soalu two varieties have been identified depending upon leaf shape size and texture, of which the ovate variety is preferred by muga silkworm than oblong (Thangavelu *et al.,* 1988).

Systemic plantation is the regular practice of som and soalu. Systemic plantation of som is more prevalent than soalu. Eight morphotypes of som have been collected, identified and raised in germplasm bank at Regional Muga Research Station, Boko, Assam. The average growth parameters biochemical analyses of leaves of all morphotypes were studied. Similarly rearing performance and post cocoon parameters were also studied on eight morphotypes. The cumulative results revealed that some morphotypes 53, 56, 54 and 55 were found palatable, superior in effective rate of rearing, cocoon production, absolute silk yield, higher filament length and raw silk recovery percentage (Siddiqui *et al.,* 2007).

Bardoloi and Hazarika (1988) after assessing the response of *Antheraea assama* to its host quality opined that leaf moisture content of the som plant regulated the rate of ingestion by the silkworm : the higher the moisture content, the higher the feeding rate and vice versa. However, dietary water acted not as a phagostimulant but as diluent of nutrients. Therefore the silkworm had to ingest more to obtain the necessary nutrients for its growth and development.

*A. assama* consume significantly more leaves of som (139.69 g , 3.62 $cm^2$/day) than those of soalu (83.30 g , 5.36 $cm^2$/day). The body weight, water content, fresh and lean dry weight of lipid of larvae fed soalu were significantly higher than those of larvae fed som though there observed no difference between them in the leaves. Qualitative variation in fatty acid composition between host plants and larvae were negligible (Hazarika *et al.,* 1995).

Among the four food plants, the conc. of K was high in som, Mg showed the same pattern but the cone. of Fe was higher in soalu followed by som. The abundance of K, Mg and Fe in som and soalu may be one of the reasons for the selection of these species as primary food plants of *A. assama* ,these major elements play a vital role in the production of silk fibers (Unni *et al.*, 1996).

Females of *A. assama* that had been reared on soalu as the larval food plant showed significantly higher pupal weight and higher oviposition rates than those on som and other food plants. The number of eggs laid was significantly positively correlated with pupal weight which was the best estimator for fecundity.

Rearing performance of muga silkworm on different food plants viz. Som, Soalu Dighloti, Gonsoroi (*Cinnamomum glaucesens*) and a natural hybrid (Dighloti x Soalu) and different seasons viz. pre-seed crop Jarua (Dec.-Feb.), seed crop Chotua (Mar.-Apr.), commercial crop (May-June), pre-seed Crop Aherua (June-July), seed crop Bhodia (Aug.-Sept.) and commercial crop Kotia (Oct.-Nov.) based on larval duration, ERR and cocoon weight revealed that Som performed best during Jarua (Dec.-Feb) crop while Soalu performed best in all other seasons, the natural hybrid was next to some during Jarua Crop while it ranked third in all other seasons. (Bhattacharyya *et al.,* 2004).

Effect of different type of diet of Dighloti, Majankari, Som and Soalu leaves on the rearing on muga silkworm was studied by Raja Ram and Samson (1998). They recorded the larval weight of 8.16 gm and cocoon weight of 4.45 gm, which were significantly higher in the treatment fed on Soalu only. The Cocoon yield (32.2% ERR) was significantly higher in the treatment when first instar on Dighloti and second to fifth on soalu were reared while silk ratio percent was calculated higher (7.16%) in the treatment when some was used.

According to Ray (2003) in West Bengal context, the performance of silk production was better on som and for seed production, soalu was better. Upon combined effect, rearing of early stage larvae on soalu and subsequent stage on som is the best combination for both silk and seed production.

**2.3 Manipulation of abiotic factors for seed production:**

Wigglesworth (1972) proposed that temperature limits between which reproduction occurs are often much narrower than the range of temperature over which the other activities of the same species remains normal. Not only reproduction but also the development and fecundity of an insect are temperature dependent.

It has been observed that muga silkworm eggs took various lengths of time to complete the embryonic development at different temperatures. Within the limits, the relationship followed almost a hyperbola. The lower the incubation temperature, the higher was the time taken to complete the embryonic development. The middle section of development curve falling between $20^0$C to $29^0$C showed straight line indicating that the rate of development was directly proportional to temperature, which was supported by hatching percentage (Barah *et al.,* 1993). Similar results have been reported from *Sitobion avenae,* an aphid species and Ali (1982) in *Patanga succineta.* Development was slowing down with further increase in temperature ($32^0$C and $35^0$C) and also indicated the possibility of being sub-lethal temperature, since the eclosion from the eggs got reduced drastically both from thermal treatments, ranging from 17 $^0$C to $29^0$C as well as room temperature. (Barah *et al.,* 1993).

In *Bombyx mori* also, with the increase in temperature from 14 to $34^0$C, the incubation period decreased from an average 12.83 days to 8 days (Upadhyay and Gaur, 2002). Chaturvedi and Upadhyay (1990) has given some idea about the effect of temperature on incubation period of *B. mori.*

The ecological factors like temperature, relative humidity and photoperiod are notably variable, therefore, for the success of the industry, the consideration of these ecological factor on the seed production sector must be taken into account (Upadhyay and Gaur, 2002).

Less intense effect of humidity on egg hatching was observed by Barah *et al.* (1993) because 65%, 75% and 85% RH produced an almost similar effect while 90% showed an adverse effect on hatching.

In *Bombyx mori* also, the increase in relative humidity caused considerable decline in the incubation period from 13 days at 35% RH to the lowest level of 9.4 days at 80% RH (Upadhyay and Gaur, 2002). Tanaka (1964) working extensively on the effects of environmental factors like humidity on larval mortality, found the high humidity not only prolonged larval moulting period but also affected physiology of the larvae thus the larvae became weak and susceptible for diseases (Benchamin and Jolly, 1986).

Photoperiod has important effect on egg hatching. In *B. mori*, Meenal *et al.* (1994) opined that for uniform and perfect hatching, eggs should be incubated under 12-18 hrs. light regime other that no light, 8 hrs. light and 24 hrs. light condition. Upadhyay and Gour (2002) observed that the variation in photoperiod regime influenced the incubation period of *B. mori* eggs. Maximum duration of the incubation was 11.77 days at 6 hrs. light a day while it was minimum of 9.55 days at 18 hrs. light a day. According to Tsurumaki (1999) all adults of *B. mori* developed under short days at low temperature ($15^0$C and $23^0$C) oviposited, only non-diapause eggs and those that developed under long days at high temperature ($28^0$C and $25^0$C) oviposited, the diapausing eggs. Incubation of eggs under complete light or complete darkness leads to irregular hatching thereby reducing hatching percentage (Chowdhury, 1984).

In *A. assama*, during short day lengths (LD 4.5: 19.5 and LD 6: 18) slowed down development rates but were not effective in inducing diapause in this insect. Females laid more eggs under short day than under long day conditions and the short day condition also showed higher hatchability (Mahanta and Goswami, 1986).

**2.4. Synchronization of moth emergence and coupling for seed production:**

Wigglesworth (1972) proposed that temperature limits between which reproduction occurs are often much narrower than the range of temperature over which the other activities of the same species remains normal. Not only reproduction but also the development and fecundity of an insect are temperature dependent. Hence, it is imperative to study the impact of temperature on different developmental stages of an insect in relation to the fecundity and viability of the eggs.

In the life cycle of the silkworm refrigeration is usually restored to one or more of the four developmental stages viz. egg ( Singha *et al.,* 1994) larva ,pupa (Tazima,1978) and moth (Tazima, 1978 ; Gowda, 1988) for various practical reasons. But strictly, the refrigeration is to be restricted to any one of the developmental stage only for avoiding deleterious effects (Jolly, 1983).

In commercial grainages the problem of synchronization of male and female moths arises. The emergence is adjusted either by changing the day of starting silkworm rearing or by refrigerating the eggs or cocoons or moths in cold storage .The usual practice is to refrigerate the male moths for a maximum period of 7 days and female for 3 days at $5^0$C (Tazima, 1978 ; Jolly, 1983). The male moths can be refrigerated before or after copulation, which can be reused for further copulation (Benchamin *et al.*, 1990). Prolonged refrigeration of virgin female moths and its subsequent effect on egg production has also been studied (Singh *et al.,* 1994).

The mating capacity of male moths of bivoltine breeds and hybrids was studied by Benchamin *et al.* (1990) under repeated mating (six times) in a day and on six consecutive days with rest at $5^0$C of male moths ,between mating Fecundity in multivoltine female percent was not influenced by different male parents .Other characters ,effective rate of mating (87.4 to 96.6%), laying yield (84.2% to 96.5%) and fertility (96.5 to 98.1%) differed significantly among different crosses and between pure breeds and hybrids as male parent. Differences between repeated mating in a single day and on consecutive days, in terms of effective rate of mating (95.8 vs. 90.0%), laying yield (85.6 vs. 93.4%) and fecundity (452.1 vs. 418.0) were found significant. All characters studied decreased significantly as the number of mating increased. The bivoltine hybrids could be used as male parent with added advantage of higher effective rate of mating, laying yield and fertility over pure breeds.

Singha *et al.* (1994) while studying the impact of refrigeration of bivoltine female moths on fecundity, viability and hatchability of eggs observed that the fecundity in general has not shown any significant difference after refrigeration of female moths up to

$6^{th}$ day as compared to control .Similar observations were also made by Tazima (1962) ; Ayuzawa *et al.* (1972) and Jolly (1983) when virgin female moths were preserved at low temperature ( $5^0$C ) for three days. Gowda (1988) described that fecundity is reduced significantly after 3 days of refrigeration of female moth. In winter season the fecundity was significantly less after refrigeration of female moths from 1 to 6 days as compared with control (Singh *et al.*, 1994). However, Singh (1992) has described rich laying in winter seasons after 72 hrs refrigeration of the female moths and also observed comparatively better hatching in winter season after 72 hrs refrigeration of female moths .Singh *et al.* (1994), however, opined that the virgin female moths of bivoltine races can the preserved at low temperature ($5^0$c) as and when required for 6 days to synchronize the mating in summer and rainy season. Such a practice is not advisable in winter season even for one day. Narasimhanna (1986) reported that even four hours coupling was sufficient in mulberry hatching and he developed suitable technique to get better quantity seed in mulberry silkworm.

At the outset of the emergence season, the males of Indian tropical tasar silkworm, *Antheraea mylitta* Drury, outnumbered the females and towards the end of the season, the trend it was reversed. As at lower temperature, the longevity of insect increased, especially in non-feeding adults (Clerk and Rockstein, 1964 and Ojha *et al.* 1996) explored the possibility of preserving males to be used for mating, if possible, several times. The effect of such refrigeration was analyzed by using performance indicators such as egg laying coefficient, mating efficiency and hatching percentage. Mating percentage and egg laying coefficients were statistically similar in the females coupled with 4 x males kept in refrigerated condition and at room temperature. Eggs obtained from females mated with refrigerated 5 x and 6 x males hatched, though at a lower percentage (48.45% in 5x and 41.88% in 6x males). Hatching was negligible after mating with refrigerated 6x and non-refrigerated 5x males. During scarcity of males in large scale of grainage, about 40 to 50% of eggs could be utilized if males were refrigerated between pairings and effectively used five or six times. Ojha *et al.* (1996) further opined that males of *A. mylitta* could be utilized for mating upto 8 times through refrigeration.

In non refrigerated *A .mylitta* moths, the egg laying co efficiency registered a declining trend when the same male was used repeatedly and this substantiate the fact that fecundity enhancing substances (FES) get depleted after every mating (Ravi Kumar *et al.,*1993).

Jolly (1987) reported that six hours coupling duration is better to get maximum hatchability in case of *Antheraea mylitta* eggs.

Maurya and Mishra (1993) carried out studies to evaluate the optimum coupling duration to get maximum fecundity and hatchability in oak tasar silkworm *Antheraea proylei*. The duration of coupling taken into consideration was one, two, three and four hours and compared with six hours coupling as control as it is the normal practice adopted in oak tasar grainage. The average no. of eggs recorded per female were 128 ± 6.06, 140 ± 10.92, 132 ± 8.07 and 138 ± 5.71 respectively and in control 119 ± 3.22 eggs only. The hatching percentage was observed which ranges between 55-69% and in control, it was 59%. As per the results obtained, there was no marked difference in the fecundity and hatchability percentage at different hours of coupling duration. So they concluded that two hours of coupling duration was sufficient instead of six hours to obtain optimum fecundity and hatching of the eggs. Mating, fertility and survival probability in male moths of *Antharaea mylitta* declined significantly with advancing age. Virgin males kept in dark showed better survival probability and survive upto 9 days where as, virgins in natural condition survived up to 8 days. Mating has a significant effect on survival of males .Regular mating leads to a lesser degree of survival than other groups .The survival probability observed was in the order of mated regularly < mated alternatively < mated on day 0 < mated on day 1 < mated on day 2 < control < virgin kept in dark. Mating possibility and fertility in the male moth declined to zero after four natural mating. Irrespective of mating status, the mating success and fertility declined with aging. Natural mating was not effective at the age of day 4. In the single mating, both mating success and fertility were considerably low when mating was effected on day 2. Weight of male moth declined significantly with advancing age but it was more pronounced in mated ones (Rath, 1998).

Such exercise was tried in *Antheraea assama,* muga silkworm for the first time by Barah and Sahu (2003). It revealed that duration of mating did not play any role in oviposition as the fecundity per female did not show any variation and remained at par with control. But the hatchability was significantly affected at 1, 2 and 3 hrs duration of mating. However, 4 hours mating duration and above did not effect the hatching performance, hence minimum 4 hrs mating duration was found optimum for successful fertilization of all the eggs of the female. Coupling aptitude of the male moths was decreased gradually with age. Beyond 24 hrs of age, coupling aptitude significantly deteriorated linearly in comparison to control showing only 32.5% natural coupling at the age of 96 hrs (4 days). All the females laid almost equal number of eggs in each treatment. Hatching performance of the eggs also decreased linearly with the age of male moths showing significant variation with control beyond 24 hours of age. However, the coupling aptitude (hatching) were not affected up to 24 hrs of age of the males. Coupling aptitude of male moths remained indifferent from control (95%) even is second mating (90%) when 4 hrs. intermittent rest period was allowed to the males in between the pairings. But 6 to 8 hrs. intermittent rest in between the pairings resulted in significant decrease per fall of coupling aptitude in second mating, which may be due to aging of the male moths. Coupling aptitude dropped down to a significant level in the 3$^{rd}$ mating irrespective of the period of rest. It was observed that oviposition had no relation with number of mating as well as with the period of intermittent rest of the male moths. They further mentioned that allowing 4 hours intermittent rest in between the pairings, even in the 3$^{rd}$ mating, above 60% hatching could be assured.

**2.5. Characterization of Cocoon for selection of seed cocoon:**

The correlation of female pupal weight with fecundity was studied in bivoltine silkworm *Bombyx mori* L. Positive and highly significant correlation existed between female pupal weight and fecundity (Sharachary *et al.* 1980; Gowda *et al.* 1988 and Shaheen *et al.,* 1992). According to Nagalakshmamma (1987) and Gowda *et al.* (1988) male pupal weight has no impact on fecundity in *Sanmia cynthia ricini*, a preliminary study was done by Nagalakshmamma (1987) who gave an indication about the female pupal weight to influence the fecundity irrespective of any dimension of male pupae used in mating after emergence. In females of eri silkworm the number of eggs laid was correlated with the pupal measurements like pupal weight, body length and body width. All the measurements considered were found to be genuine estimators of the fecundity, however, the pupal weight was the best estimator of fecundity.

Miller *et al.* (1982) reported significant correlation between the number of mature eggs and total eggs, concluded that the pupal weight was the best estimator of number of matured eggs in *Antheraea Polyphemus* (Gamer).

It is evident that a correlation between the dimensions of the pupae of *Antheraea mylitta* and egg laying exists and the pupae with larger dimensions is $10.5 \pm 0.49$ g weight, 4.5 cm abdominal girth and 3.5 cm abdominal length by larger number of eggs as compared to those with shorter dimensions with the observation that (Badhera,1992). Pupal weight was the best estimator of fecundity. Detailed works had been done by Dubey *et al.* (2005) on selection of seed cocoons in *Antheraea mylitta* and its correction with fecundity and hatching. Pupal weight has been identified as one of the estimator for selection of seed cocoons. Pupae were segregated sex-wise under different weight groups ranging from extreme high to extreme low. The study indicated that the medium weight range of male (8.50-10.59 g) and female (10.54-14.29 g) pupae were distributed abundantly in the normal population of first crop harvested seed cocoons. The study also indicated that this medium weight range of both male and female seed cocoons overlaps each other comes in the way of selecting the male and female seed cocoons for the grainage. The emerging adults from various pupal weight groups were crossed reciprocally in all possible combinations .The female pupal weight was observed as an estimator for fecundity, whereas male pupal weight did not contribute any significant influence either on fecundity or on hatching.

Correlation and regression studies between pupal weight and fecundity of muga silkworm, *Antheraea assama* was also done on different food plants. According to Barah *et al.*, (1989) females recorded on soalu showed significantly higher pupal weight and higher oviposition rates than those on som and other secondary host plants. The number of eggs laid was significantly positively correlated with pupal weight which was the best estimator for fecundity. Yadav *et al.* (1992) while observing the association of fecundity with cocoon weight, pupal weight and shell weight of muga silkworm during two commercial broods reported that cocoon weight, pupal weight and shall weight were positively correlated with fecundity.

**2.6. Short-term cold preservation for seed production:**

The structure of egg is the determiner of the pattern of development (Boswell and Mahowald, 1985). The structure and development of eggs in various insects have been described by many workers (Engelmann, 1970; Jura,1972; Balinsky ,1981). The first detailed study on the development (embryogenesis) of silkworm *Bombyx mori* egg was carried out by Toyama (1902). Since then embryological studies were confined especially to solve the practical problems, such as identification of suitable stages for refrigeration of early embryos and the initiation, continuation and termination of diapause in order to develop an effective system for long-term cold storage of silkworm eggs.

Takami (1969) was apparently the first who published a review on the embryogenesis of the silkworm, *Bombyx mori*. Morphological changes occurring during different embryonic stages in the silkworm, *B. mori* were described (Nakada, 1932; Takami and Kitazawa, 1960). In these studies, morphological changes of embryos were used to assess their long term survival. Moreover, the tolerance of eggs to cold storage varies with the stages of embryonic development besides genotypes because of adaptation phenomenon, metabolic changes and also genetic variations etc. (Salt, 1961 ; Rockstain, 1974 ; Sander *et al.,* 1985 and Sonobe *et al.,* 1986) and hence the recent studies on silkworm eggs become more concerned with physiology, biochemistry and metabolic activity associated with termination of diapauses (Yaginuma *et al.,*1990;Yamashita and Yaginuma, 1991) for effective handling.

It is believed that for any particular individual there is a precise limit to the time during which it may survive exposure to a particular lethal temperature and this may be true for any stage of the life cycle of that individual (Andrewartha and Birch, 1954). Watters (1967) found that eggs of *Trilobium confusum* less than four hours old were readily killed on exposure at $5^0$C or $10^0$C for five days but older eggs become progressively more resistant to these temperatures and nearly 30% of three to four day old eggs survived a 15-day exposure. As it is a already established from similar studies of other workers (Howe, 1967) that the first stages of embryonic development of insect eggs are very crucial and sensitive to low temperature.

Dutta *et al.* (1972) determined the effect of preservation at low temperature (5-7 $^{0}C \pm 1\ ^{0}C$) on the hatching of multivoltine silkworm *Bombyx mori* L. eggs following incubation for different periods. Results indicated that the eggs incubated for 1.5 days at room temperature are most suitable for long time preservation. In general, eggs incubated for shorter periods are able to withstand preservation for larger periods in comparison to eggs incubated for a larger period and then subjected to low temperature preservation .The results indicate different scheduled of preservation which can successfully be utilized by the Sericulturists where postponement of hatching is needed for synchronization or solving technical difficulties related to distribution of layings.

Ibohal *et al.* (1987) reported that oak tasar eggs (*Antheraea proylei* ) can be cold stored up to 30 days without any adverse effect on hatching ,effective rate of rearing ,cocoon weight ,shall weight and reliability. Pandey *et al.* (1992) has reported that oak tasar silkworm eggs can be stored in refrigerator only for 17 days. Pandey *et al.* (1992) observed that they can be stored up to 10 days without any adverse effect on hatchability. After 20 days of cold storage less than 1% embryonic death occurred between 7 and $11 \pm 2^{0}C$ where as after 30 days upto 3% embryonic death occurred at the same temperatures. The embryonic death was relatively more up to 6.46% at $5 \pm 2^{0}C$ upto 30 days .But after 40 days of cold storage only 9.8% embryonic death occurred at $5 \pm 2^{0}C$ where as upto 15.5% embryonic death occurred between 7 and $11 \pm 2^{0}C$. It was further observed that eggs of only one day age could not survive even for 10 days of cold storage at $5 \pm 2^{0}C$. Thereafter, from the second day onwards the growth of the developing embryo can be temporarily arrested by cold storage up to 10 days without any loss and upto 30 days with less than 2% loss and upto 40 days with less than 10% loss. Effect of refrigeration on the hatching of *Antheraea Proylei* eggs was studied by Biren Rana *et al.* (2002) also to determine the suitable age of eggs, temperature and duration of refrigeration in order to delay hatching. 24, 48 and 72 hours old eggs were subjected to cold storage at 0, 5, 10 and $15^{0}C$ for a period of 5-35 days, than incubated at $24 \pm 2^{0}C$ and the hatching was recorded. Observation of eggs at $0^{0}C$ revealed maximum adverse effect on the hatchability of 24 h old eggs with all durations of storage while at $15^{0}C$ irregular hatching started beyond 15 days of refrigeration period. The adverse hatching in eggs of all ages was highest at $10^{0}C$ with more than 60% hatching upto a period of 20 days and declined thereafter. The age of eggs and the period of refrigeration at four different temperatures were observed to have a significant effect on the percentage of hatching.

The effect of temperature on the incubation of *Antheraea yamamai* eggs was observed by Zhang *et al.* (1998) who reported that the egg embryos did not develop below 5.5$^0$C, partly incubated between 7-10$^0$C and the incubation became rapid correspondingly with the rise of temperature from 7 –30$^0$C.

Nangia and Nageshchandra (1988) recorded good hatching in the eggs of *Samia cynthia ricini* (laid within 72h) upto a period of 15 days when refrigerated at 0$^0$C. They further observed that storage of eggs at 5-10$^0$C for 5-10 days gives the highest effective rate of rearing. Vishwakarma (1982-83) observed that at 7$\pm$2 $^0$C, 3days old eggs of *P. ricini* in summer and 5 days old eggs in winter are more resistance to cold than at 3 1$^0$c (Govindan *et al.*, ,1980 ) where refrigeration beyond 5 days of ate has an adverse effect on hatching.

In *Antheraea assama* an attempt has been made by Biswas and Ray (2007) to avoid rearing during mid June to mid August reflecting poor supply of seed during main commercial season (Oct. – Nov.) by refrigerating the cocoons from previous commercial crop (May –June). From the results it can be said that 15 days cocoon preservation can delay the moth emergence for 10 days, adult moth preservation for 5 days and eggs after 24 hrs incubation for 21 days.

Sengupta and Singha (1974) studied the refrigeration of muga seed cocoons at 4$\pm$1$^0$C for 5 to 50 days and found gradual reduction after 40 days of preservation. Subha Rao and Choudhury (1976) also observed the effect of preservation of muga cocoons at low temperature. According to them muga pupae could be preserved at 2.5 to 5$^0$C for more than 75 days in winter and 30 days in summer to delay in the moth emergence. Thangavelu *et al.* (1985) preserved muga seed cocoons at 5 and 10$^0$C and at higher altitude (2590m). They obtained better results after 3 months of preservation. Several other studies (Choudhury *et al.* 1980 and 1985 and Bora *et al.* 1980, 1990 and 1992) also revealed that refrigeration of muga seed cocoons at 5 to 12$^0$C for 10 to 120 days gave satisfactory results in terms of moth emergence, pairing, fecundity and hatchability. In a study on preservation of muga seed cocoons of autumn crop at 8$\pm$1$^0$C and 60-70% RH, could delay moth emergence till 60-80 days as compared to 30 days in control Khanikor and Dutta (1998). A preservation for 35 and 40 days could be suitably utilized for 'Chotua' crop (Feb.-Mar.) by overcoming the hazardous winter crop (Dec.-Feb.). Khanikor and Dutta (1998) further opined that the moth emergence could be delayed from a normal period of 28-30 days to 60-120 days in case of autumn cocoons, 40-45 days to 80-100 days in the case of late autumn cocoons and 14-18 days to 30-42 days in the case of spring cocoons .Based on reproductive parameters of preserved individuals ,growth and cocoon parameters of their progenies suitable results were obtained from the lots preserved for 35-45 days in commercial autumn crop, for 45-60 days in late autumn crop and for 20-30 days in commercial spring crop. Declining trends in all the parameters were observed beyond these periods of preservation of seed cocoons has been taken by Sengupta *et al.* (1995) where ten day old cocoons were kept at $7^0$C and $10^0$C showing safe preservation at 10 $^0$C for 45 days without affecting their reproductive physiology.

Singha *et al.* (1998) opined that the longest embryonic stage which occurred at 36 hours of oviposition during May and 114-126 hours of oviposition during November exhibited resistance to low temperature treatment without impairing hatching in muga silkworm.

Seed cocoons of autumn crop of muga silkworm were preserved at low temperature regimes of $5 \pm 1^0$C, $7 \pm 1$ $^0$C and $9 \pm 1^0$C for 30, 40, 45, 50, 55, 60, 65, 70 and 75 day. Declining trend was observed in all the parameters, viz. moth emergence pairing, fecundity and hatchability. Moth emergence could be delayed up to 60-120 days against 28-30 days in control. Better results were obtained in 35, 40 and 45 days of preservation compared to the rest. Muga seed cocoons of autumn crop can be preserved effectively for 30-45 days to delay moth emergence to avoid the prolonged Jarua crop and to synchronize with the subsequent main seed crop (Chotua) for raising the commercial spring (Jethua) crop (Khanikor and Dutta, 2006).

Finally, Upadhayay and Pandey, (2000) opined that the weight of cocoon obtained from the long-term refrigerated eggs was of low grade but the cocoon obtained from the short-term refrigerated eggs was of comparatively better in weight, hence suggesting avoidance of long-term preservation as far as possible.