Original Research Article

Morphological Variation of *Antheraea assamensis* Helfer upon semi-domestication: A study on Rearing and seed production performance

Abstract

Muga silkworm is multivoltine and primarily feeds on two primary food plants Som (*Persea bombycina* King. syn. *Machilus bombycina*) and Soalu (*Litsea polyantha* Kost. syn. *Leatsia monopetala*). Muga silkworm *Antheraea assamensis* (n=15) is a semi-domesticated silk moth mentioned in literature as early 1662 BC. In its annual life cycle there are six crops of cultivated muga silk worm whose nomenclature is based on the local Assamese names of month. By virtue of the narrow ecological distribution of host food plant *Antheraea assamensis* is confined to only Assam and North East state of India. Empirical observations show that the population is declining due to depletion of natural habitat and lack of genetic variability among population. The wild muga silkworm which is tri or tetra voltine in nature is also found nearby forest area of human habitat in different host plant in N E state of India. A stock of wild muga silkworm collected from the nearby area of Nongpoh and Mendipathar Meghalaya. reared and maintained in muga farm and grainage activities of both wild and cultivated muga were conducted for comparative study. Details of morphology, economic characters and disease occurrence of wild and cultivated muga was studied for future improvement and breed development and also for future strategy of muga silkworm. The morphology of wild muga silkworm are little variant than cultivated muga and economic characters *i.e.* fecundity of wild muga was recorded 220 - 227 nos. in against the fecundity of cultivated muga observed 120-170 nos. and one gram total numbers of egg contain 120 -135 nos in wild muga in against 134 -145 nos.in cultivated muga. Average cocoon weight wild muga cocoon is ♂= 5.80 g & ♀= 6.60 g in against average cocoon weight ♂= 5.10 g & ♀= 5.54 g in cultivated muga silkworm. Average shell weight ♂= 0.51 g & ♀= 0.60 g in wild muga silkworm and average shell weight ♂= 0.43 g & ♀= 0.51 g in cultivated muga silkworm were recorded. Study revealed that the fecundity, weight of eggs, cocoon weight and shell weight are higher in wild muga silkworm than cultivated muga silkworm. Thereafter it is found that only protozoon disease was reported in wild muga silkworm and other disease *i.e.* Flacherie, Grasserie, Muscardine was not reported in

wild muga silkworm. The mono race muga have no high yielding breed or hybrid and hence breeding programme between wild and cultivated muga may be carried out to get more heterosis, vigourity and yield enhancement.

Keywords: Wild, cultivated, Muga , Silkworm , Morphology, Rearing, Seed production

Introduction:

Muga is the golden yellow glistening silk, which is the product of the cultivated muga silkworm (*Antheraea assamensis* Helfer). Assamese word "muga" which indicates the amber (brown) colour of muga cocoon. It occupies a distinct socio-ecological niche amongst the folks of North East India, especially Assam. Demographically, the muga silk is cultured indigenously in the North East region of India and 95% of this silk is produced in state Assam only. It is popular for its natural golden colour, glossy fine textures and durability. Muga silkworm *Antheraea assamensis* (n=15) is semi-domesticated silk moth mentioned in literature as early 1662 BC. Muga silkworm is multivoltine and basically feeds on two primary food plants Som (*Persea bombycina* King. syn. *Machilus bombycina*) and Soalu (*Litsea polyantha* Kost. syn. *Leatsia monopetala*). In its annual life cycle there are six crops of cultivated muga silk worm whose nomenclature is based on the local Assamese names of month. By virtue of the narrow ecological distribution of host food plant *A. assama* is confined to only Assam and North East state of India. Empirical observations show that the population is declining due to depletion of natural habitat and lack of genetic variability among population.

Methodology:

Collection of wild muga:

The maturation of wild *A. assamensis* larvae was observed in Mawsyntai village forest in the Rhi Bhoi district of Nongpoh, Meghalaya, India. To protect the muga population, a stock of wild muga silkworm was collected from the nearby area of Nongpoh, Meghalaya. The wild muga silkworm which is tri or tetra voltine in nature is also found nearby forest area of human habitat in different host plant in N E state of India (Tikader, 2013). After collection wild muga silkworm was reared and maintained in the muga farm and comparative study of morphology,

economic characters and disease occurrence of wild and cultivated muga was studied, which may help for further study for improve genetic improvement for positive impact of breed development and seed production and also for future strategy for stability of muga silkworm.

The semi-domesticated morph of *A. assamensis* was maintained in Muga Eri Silkworm Seed Organization, P3 Seed station Nongpoh, Meghalaya. The larvae were fed on *Persea bombycina* (King ex Hook. Fil.) Kosterm.

Rearing & Grainage:

The morphological study of wild and cultivated muga was done during rearing and grainage activities. The mature ripened worms were collected and placed in mountages to spin cocoon under indoor condition. Approximately 6-7 days after completion of spinning, the muga cocoons were harvested. The harvested wild and semi-domesticated cocoons were preserved for seed production for comparative analysis. The morphology of muga worms and economic characteristics like cocoons character, pupal character, egg character *etc*. of wild and cultivated muga were analyzed. Throughout the rearing and grainage period, the meteorological data at MESSO, CSB, P3-Nongpoh, Meghalaya, India was recorded as briefed in Table 1 .

Table 1. Temperature, Relative humidity and rainfall recorded for the year 2020 at MESSO, CSB, P3 Nongpoh, Meghalaya, India

Month	Rain fall (mm)	Rainy day's	Outdoor				Indoor			
			Temp ^{0}C		RH%		Temp ^{0}C		RH%	
			Min	Max	Min	Max	Min	Max	Min	Max
January	7	1	05-11	18-23	41-88	84-94	12-15	17-20	39-70	70-94
February	17	3	05-13	20-25	47-75	84-95	11-17	17-21	43-61	59-88
March	330	5	11-16	21-30	38-75	68-94	16-21	20-25	33-66	58-88
April	600	8	13-71	22-32	31-79	67-95	18-22	21-28	33-64	52-94
May	764	15	15-20	27-30	43-90	72-95	18-23	23-29	43-78	68-95
June	1106	19	18-24	28-33	61-90	86-95	21-25	24-30	70-87	83-96
July	1160	26	22-24	28-33	58-90	75-95	24-26	28-9	55-91	45-95
August	1510	20	22-25	27-36	44-87	71-92	24-27	24-30	62-86	72-96
September	930	20	22-24	27-35	61-83	82-95	25-27	27-32	58-82	78-91
October	1691	17	19-23	26-35	57-90	86-95	23-26	27-31	56-83	78-91
November	479	3	12-21	25-31	40-83	82-95	20-25	28-31	48-82	64-92
December	--	--	06-14	19-26	34-75	75-94	15-21	28-31	51-74	65-84

Result and Discussion:

Morphological characters of wild and cultivated muga silkworm:

The morphological characters of wild and cultivated muga was observed. Wild muga is tri or tetra voltine in nature, it completes 3-4 life cycle in year (Kakati *et. al. 2006*). Cultivated Muga exhibits Multi voltine *i.e.*, complete 5-6 life cycles in a year. (Chakraborty,2004).

Young Larvae:

During rearing, the larval characteristics was observed to be similar in wild and cultivated muga, newly hatched larva is characterized by prominent black, inter-segmental markings over the yellowish body with brown head. After the first moult the body turns greenish, while the head remains brown. Yellowish colour body larvae can also be seen. .in the third moult larvae which becomes deep greenish body colour with purple tubercle.

Mature Larvae:

The matured muga larvae are deep green in colour with prominent tubercle and last segmental spot. Also, some of the matured wild muga are larger and dark green colour with prominent tubercle and last segmental spot. The male and female cultivated muga are almost same in colour, but female one little larger in size. In cultivated muga the male and female worms are almost same in colour, but female are larger in size. The larvae are eruciform in type and 3 thoracic segments and 10 abdominal segments with 3 pairs of thoracic legs and 5 pairs of pseudo legs on 3^{rd}, 4^{th}, 5^{th}, 6^{th} and 10^{th} abdominal segments. The latter one is called as claspers. Marginal lines of mature worms are distinguishing prominent yellow in female and blackish in male wild muga. The type of mouth parts of the muga silkworm is biting and chewing type. Wild muga silkworm is robust, very sensitive and larger size in compare to cultivated one. Cultivated muga are less vigourity in compare with wild muga.

Cocoon characteristics:

The cultivated muga cocoon shows a brighter golden brown hue than the wild cocoons. The cocoon is elliptical, closed, reelable. The wild cocoon is slightly compact with elongated strong peduncle. The cultivated cocoon is slightly softer than wild muga cocoon with a vestigial peduncle.

Moth characteristics

The male moth is smaller than female. The body length measures 3.2 cm in wild muga and 3.0 cm in cultivated muga, in width length i.e., from wings tip to tip, measures wild muga measures 14.0 cm and cultivated muga measures 13.0 cm in width. Similarly the female has body length of 3.6 cm and width wings tip 16 cm in wild muga and 3.5 cm and width wings tip 15 cm in cultivated muga. The abdomen of female is bigger than male muga moth in wild and cultivated muga. Wild muga male moths are deep brown and pale brown in colour and females are pale brown and yellowish in colour the hue in cultivated moth is darker than wild. The anti-median line and the oblique line possess pale white boarder on its inner surface whereas the post median line is bordered by two nos. of white lining in wild moth and by single pale white lining in cultivated moth on either side in male and in female. The wild and cultivated muga moth has a pair of bipectinate antennae.

Fecundity and egg characteristics

The Fecundity of wild muga recorded 220- 227 nos. On an average consisting of 120 - 135 nos in one gram. The Fecundity of cultivated muga was observed to be 120-170 nos. based on the climate and season. In one gram, total numbers of egg was 134 -145 nos. The eggs of wild muga moths are streaky and deep brownish appearance. The follicular imprints consist of a single pattern with oval main cells. The eggs of cultivated muga moths have less streak and brownish in appearance.

In wild muga, embryonic developmental period take place 9 -13 days in suitable climatic condition and larvae hatch out after complete development. In low temperature in winter embryonic development may extend up to 18-20 days. In cultivated muga after complete development of embryo, Larvae hatch out 9-10 days after oviposition. In low temperature in winter embryonic development may extend up to 12-14 days.

The morphological and economic characteristics of wild and cultivated muga worms like cocoons character, pupal character, egg character *etc*. of wild and cultivated muga were analyzed and briefed in detail **Table 2**. To observe the morphological variation and other economic characters the rearing, seed production activities and disease occurrence of wild and cultivated muga were studied and comparative data were recorded. The morphology of wild muga silkworm are little variant and economic characters *i.e.* fecundity of wild muga was recorded 220 -227 nos. in against the fecundity of cultivated muga observed 120-170 nos. based on the climate

and season. In one gram total numbers of egg contain 120 -135 nos in wild muga in against in one gram, total numbers of egg found 134 -145 nos. Therefore, it is found that wild muga are better performer than cultivated muga in case of grainage character. Average cocoon weight wild muga cocoon is ♂= 5.80 g & ♀= 6.60 g in against average cocoon weight ♂= 5.10 g & ♀= 5.54 g in cultivated muga silkworm. Average shell weight ♂= 0.51 g & ♀= 0.60 g in wild muga silkworm and average shell weight ♂= 0.43 g & ♀= 0.51 g in cultivated muga silkworm were recorded. Study revealed that the fecundity, eggs weight, cocoon weight and shell weight are higher in wild muga silkworm than cultivated muga silkworm.

Table 2 Observation of morphological and economic characteristics of Wild and cultivated muga during rearing and grainage period.

Type	**Wild male**		**Cultivalted male**		**Wild Female**		**Cultivalted female**	
Place	**Nongpoh**	**Mendipathar**	**Nongpoh**	**Mendipathar**	**Nongpoh**	**Mendipathar**	**Nongpoh**	**Mendipathar**
Larval weight (gm)	12.19	12.2	9.79	9.92	13.88	14.87	10.21	11
Cocoon weight (gm)	5.8	8.1	5.1	7.22	6.6	9.9	5.54	7.14
Shell weight (gm)	0.51	1	0.43	0.41	0.6	1.15	0.51	0.6
Pupal weight (gm)	5.29	6.22	4.67	4.2	6	8.78	5.03	6.8
Pupal Diapause (days)	96	92	30	27	100	96	33	35
S.R. (%)	12.79	13.8	9.6	10.6	11.88	11.5	9.2	9.69
Fecundity					224	193	165	170
No of egg/gm					128	130	140	143

*The f-ratio value is 22.13289. The p-value is < .00001. The result is significant at p < .01.

**Two-tailed P-value=0.0455.The result is significant at p < 0.05.

Diapauses behaviour of muga silkworm:

Wild muga silkworm hibernate in pupal stage in the Soalu (*Litsea monopetala*) plant during winter in time of shortage of leaves, however the muga silkworm feed on Som leaves do not hibernate (Kakati,1991,1993). Silkworm species of *Antheraea* undergo diapauses in the pupal stage, except *A. yamami*, which undergoes diapauses in both the egg and pupal stages (Jolly *et al*.;1973; Ahsan *et al*.,1976; Choudhury,1981; Kato & Sakate,1982; Khanikar & Dutta,1997,1998,2000). Wild muga undergoes pupal diapauses and uni - voltine character (Choudhury, 1981; Thangavelu & Bhagawati, 1984). Cultivated stock of muga silkworm, which has been semi-domesticated, is now adapted as multivoltine character (Negi *et al*.) and in a year 5-6 brood may complete in different crop period. During the winter season, crop cycle complete 80-84 days with 36-40 days larval period and 40 - 42 days pupal period without diapauses.

Disease occurrence of wild and cultivated muga silkworm:

Diseases are one of the major problems encountered by the muga rearers and muga silkworm crop loss. The crop loss by diseases is alarmingly due to high humidity and fluctuation of temperature. The average crop loss due to diseases in muga as a whole 35-40 % crop loss occur due to diseases and pests. The incidence of muga silkworm diseases is greatly influenced by rearing practices, frequency of cropping, general hygiene, seed quality, environmental conditions favourable for pathogen build up and spread. The silkworm diseases are best prevented than cured. There are no curative measures for any of the silkworm diseases and have to be only prevented by proper disinfections and maintenance of hygiene. Strict monitoring of disease must be made right from seed production till cocoon harvest. Muga silkworms also like any other varieties of silkworms suffer from protozoan, bacterial, viral and fungal diseases.

During the study of this experiment disease incident was not observed. But it was reported that wild muga were collected from perennial food plants of foothills near by human habitat or other area have found protozoon disease only, Other disease like Flacherie, Grasserie and Muscardine disease were not reported in wild muga silkworm.

Pebrine disease:

Protozoan disease, Pebrine is one of the dangerous diseases spread generation to generation in cultivated muga silkworm. It is caused by *Nosema antheraea* in muga silkworm of the family *Nosematidae*. The disease may be occurs from mother moth through eggs, by contact with diseased worms and through ingestion of contaminated food plants. There are two stages of

pebrine in its life cycle *i.e.* the Spore stage and Vegetative stage. The disease is more acute in young stages than later stages. ***Early stage infection***: Larvae appear normal, microscopic examination may reveal presence of pebrine spores. In advance infection cases the larvae cease to feed and show irregular growth and moulting. ***Later stage infection***: Silkworm larvae loose appetite, varies in size, retard in growth. Under condition of heavy infection, black spots appear on the skin (**Fig. 3**) as the parasite invades the hypodermal cells of the skin (Jolly *et al*., 1975 & J P Barua 2021). These cells die here and there showing melanosis and appear as dark pepper like spots. ***In the moth stage*:** Infected seed cocoon, moth, grainage appliances, muconium and moth scales, grainage dust. Examination of the diseased eggs, larvae and moth under the microscope shows numerous elongated oval shinning spores of pebrine.

Flacherie disease:

Flacherie may be caused by association of Virus or Bacterial infection. A syndrome associated with infectious flacherie is Densonucleosis virus (DNV), Cytoplasmic polyhedrosis virus (CPV) and several types of Bacteria. Infected larvae lose appetite, become sluggish and grow slowly. The larval body loses elasticity and becomes soft and Diarrhoea and vomiting are associated. The larvae does not feed after moulting, the body shrinks. The larvae hold to branches with the anal claspers, finally hangs downward and die **(Fig. 3).** The symptoms of this disease is not always uniform and vary according to the time of occurrence, the bacteria involved and other conditions. Flacherie is more prevalent during high temperature, high humidity, poor quality leaf, overcrowding, decreased gut alkalinity *etc*. The disease prevails in all seasons but is intensive during rainy summer months.

Grasserie disease:

Nuclear Polyhedrosis Viral (NPV) disease, commonly known as Grasserie or "Phularog" caused by Baculovirus. Grasserie is dangerous disease of cultivated muga silkworm spread quickly to the whole stock. The integument becomes fragile and inter-segmental region becomes swollen **(Fig. 3).** Grasserie predominantly occurs during rainy summer months, when temperature and humidity fluctuate freequently and rain water stagnent in the rearing site or humidity increased drastically. Body tissue and haemolymph get disintegrated into turbid / white fluid. Larvae hang upside down with the help of anal claspers. Microscopic examination of

turbid fluid shows large number of hexagonal polyhedral bodies. Pre disposing factors *i.e.* poor quality foliage feed and high temperature with high humidity caused Grasserie disease.

Muscardine disease:

Muscardine disease is caused by fungi *Botrytis bassiana* and *Beauvaria bassiana*. Commonly occurs during winter season when night temperature is low but day temperature remain comparatively high associated with high humidity due to foggy weather. Generally white and green muscardine diseases are found in muga silkworm. Infected larvae loose appetite and become inactive. Larvae gradually cease movement within 12 - 18 hours of infection. After death the larvae die and body gets hardened. In next 16-18 hours white encrustation appears on larval body. Within another 24 hours the whole body gets covered by white encrustation, becomes dry, Chalky and mummifies (**Fig. 3**). Pre disposing factors of muscardine disease is low temperature and high humidity.

Thereafter it is found that only protozoon disease was reported in wild muga silkworm and other disease *i.e.* Flacherie, Grasserie, Muscardine was not reported in wild muga silkworm. It is well-known that the muga silkworm is mono race and has no high yielding breed or hybrid for commercial exploitation and productivity improvement. The breeding programme between wild and cultivated muga may be carried out to get more heterosis, vigourity and yield enhancement in the new hybrid generation. Also, the hybrid population may be resistance to common muga silkworm disease other than protozoan, because of wild muga are more disease resistance to bacterial and viral infection than cultivated muga.

Conclusion:

The present study revealed that as in other Lepidoptera, there exist variations of the morphology of pre-cocoon in different stages *i.e.* larval, cocoon and moth characters in cultivated and wild muga silkworms. One-way ANOVA analysis reveals f-ratio value is 22.13289. The p-value is < .00001. The result is significant at $p < .01$. In the Two-tailed T-test, P-value=0.0455. The result is significant at $p < 0.05$. Hence, it can be affirmed that semi-domestication leads to morphological variation in *Antheraea assamensis* Helfer during rearing and seed production. Silk yield is a complex trait and is contributed by different characters to varying degree. Moreover, most of the characters are influenced by both environmental and genetic factors and the contribution of which vary for different stock (Sarkar *et.al.* 2016). The success of breeding is

largely depending on the choice of parents, mating system in designing the breeding plane followed by appropriate selection procedure of breeding material. Analysis of the growth, development and economic characters of rearing, grainage and cocoon yield of different stock of wild and cultivated muga silkworm, revealed that the superior wild muga stock may be effective parents for promising combinations with semi-domestic cultivated muga stock for development high yielding muga breed/hybrid for increasing of muga raw silk.

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Table Legend

Table 1. Temperature, Relative humidity and rainfall recorded for the year 2020 at MESSO, CSB, P3 Nongpoh, Meghalaya, India

Table 2 Observation of morphological and economic characteristics of Wild and cultivated muga during rearing and grainage period.

Figure Legend

Figure 1: Wild Muga larvae, cocoon, moth and eggs

Figure 2: Cultivated Muga larvae, cocoon, moth and eggs

Figure 3: Diseases of Muga silkworm

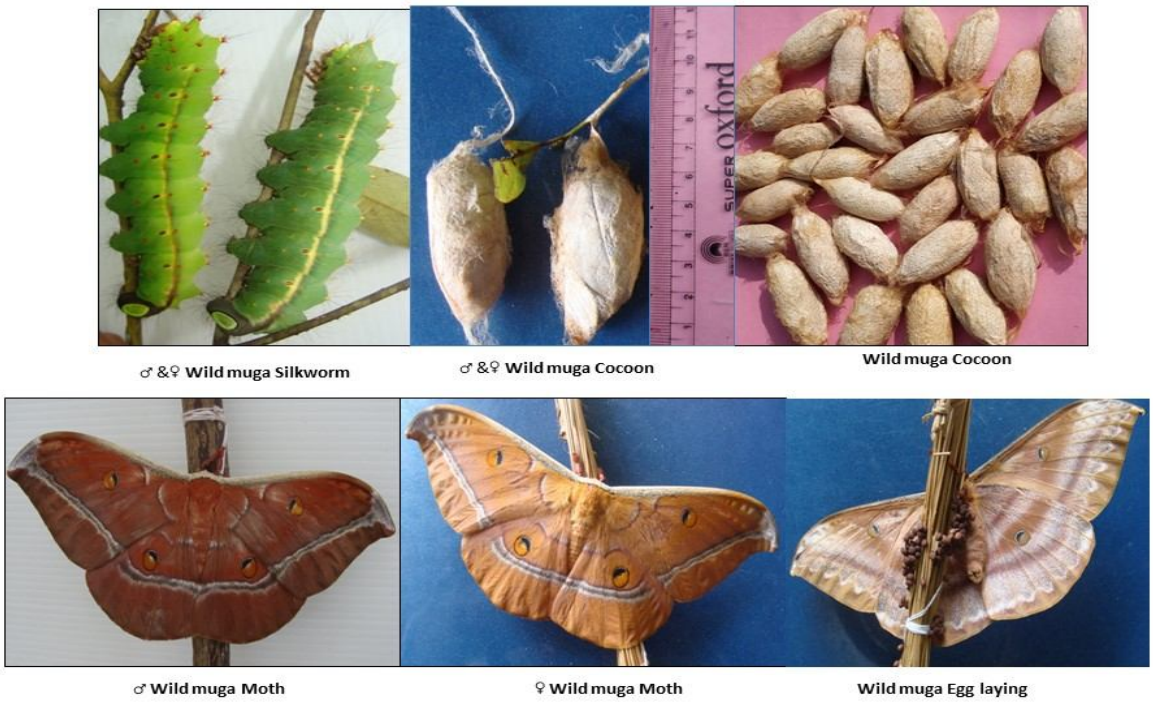

Figure 1: Wild Muga larvae, cocoon, moth and eggs

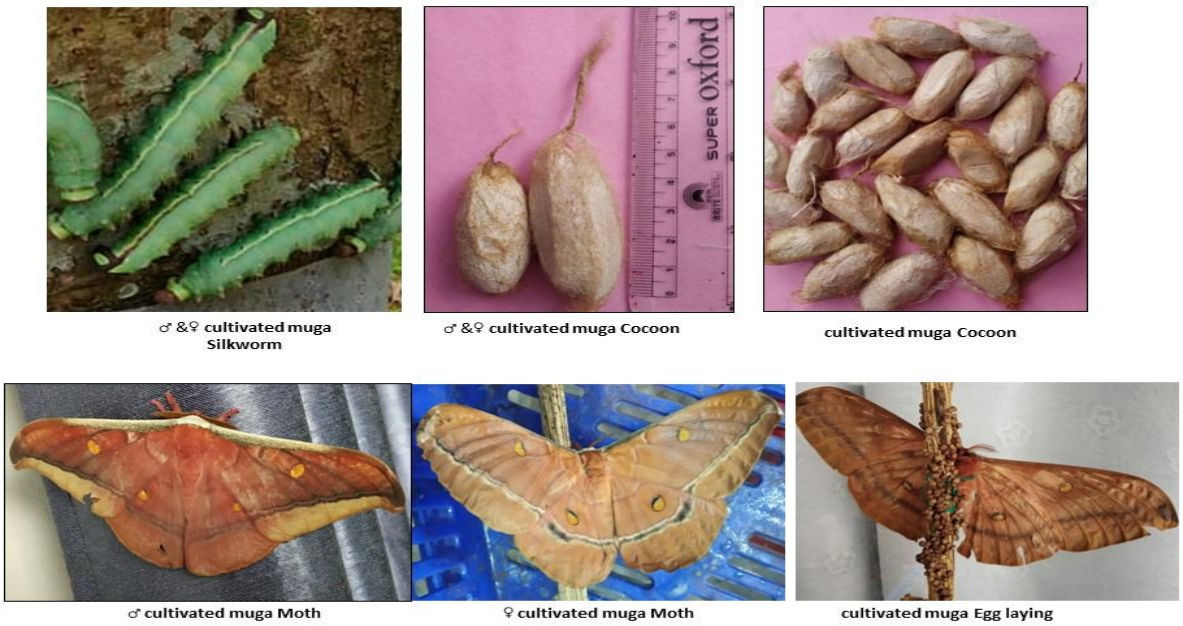

Figure 2: Cultivated Muga larvae, cocoon, moth and eggs

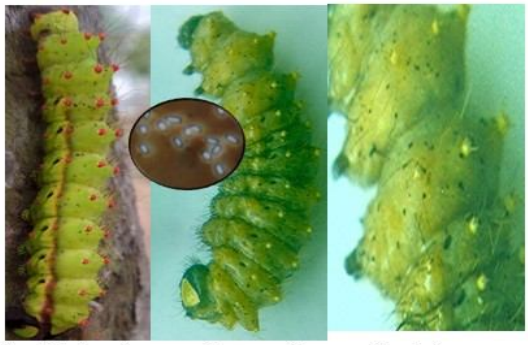

Fig: Pebrine disease of muga silkworm & pebrine spore

Fig: Flacheire disease of muga silkworm

Fig: Grasserie disease of muga silkworm

Fig: Muscardine disease of muga silkworm

Figure 3: Diseases of Muga silkworm

Fig.4 Wild female moth

Fig.5 Wild male moth