



## ***SUMMARY AND CONCLUSIONS***

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**A**mong the silkworm, diseases, the microsporidiosis (pebrine) is the most dreaded and destructive disease. It is unique being transmitted from parent to offspring through the egg. In the past the microsporidiosis in silkworm was known to be caused by *N. bombycis* alone but in recent days it is known that several microsporidian species cause the disease in silkworm. The mode of infection is primarily *per os* and transovum and transovarial. The different microsporidians infecting silkworm differ in infectivity, transmission, pathogenicity and virulence. A few of them are high in virulence and some of them transmit infection to an extent of 100% to the progeny while others either nil or as low as 1.8%.

In the present study a microsporidia infecting in Lamerin breed of silkworm a native of Northeastern part of India, was characterised. The microsporidia is in association with the Lamerin breed for past several generations without being eliminated and causing any significant harm to the breed. The understanding of the Lamerin microsporidia infecting the silkworm breed Lamerin is of great practical importance in management of pebrine disease in silkworm. Hence the present investigation was under taken to characterize the microsporidia infecting Lamerin breed of silkworm.

The salient features of the investigation and results are as follows.

- ❖ The Lamerin breed of silkworm *Bombyx mori* is a native of north-eastern state Manipur, India. It is a diapausing bivoltine breed with few features of multivoltine. The eggs are light whitish yellow turning to light brown after diapause. The average fecundity was  $301 \pm 22.26$ . The larvae were plain without larval marking, slender and bluish white. The mature larvae measured  $47.60 \pm 2.32$  mm in length and  $4.82 \pm 0.30$  mm in width. The larvae weighed 2.59 g and the larval period was 23 days. The cocoons were spindle shaped, orange yellow with high percentage of floss. The average cocoon and shell weight and silk ratio was  $1.474 \pm 0.02$ g,  $0.192 \pm 0.01$ g and  $13.06 \pm 0.57\%$  respectively. The pupae were slender brown and the pupal period was  $11.50 \pm 0.53$  days. The average pupation rate was  $91 \pm 2.21\%$  and the moth eclosion was  $89.3 \pm 2.41\%$ . The moths were active and cream yellow in color.

- ❖ The Lamerin breed silkworms were found infected with a microsporidium. The microsporidian spores were isolated and purified by percoll gradient centrifugation. Its infectivity to lamerin breed followed the principles of Koch' postulates. The purified microsporidian spores were ovo-cylindrical measuring  $4.36 \pm 0.06 \mu\text{m}$  in length and  $2.14 \pm 0.01 \mu\text{m}$  in width.
- ❖ The internal ultrastructure of spore of Lamerin microsporidium shows that the spore has polar tube in 11 coils, 2 nuclei and a posterior vacuole. The average spore coil length is  $0.066 \mu\text{m}$  and spore width is  $0.076 \mu\text{m}$ . The coil length width ratio was 1:1.15. The spore wall of Lamerin microsporidia consisted of a thick and uniform endospore coat and projection like ornamented exospore. The spore consists of a posterior vacuole, nucleus and a mushroom shaped anchoring disc.
- ❖ The Lamerin spore surface protein had five major peptide bands of molecular weight 66, 50, 31, 28 and 20 kDa as against six major peptide bands of molecular weight 64, 59, 31, 29, 27 and 20 kDa in *Nosema bombycis*. The 31kDa band of Lamerin microsporidia was of high intensity than that of *N. bombycis*. The 28kDa band is of diffused type and in *N. bombycis* 20kDa band is comparatively more intense than that of Lamerin microsporidium.
- ❖ The Lamerin microsporidian spore did not react positively with monoclonal antibody based agglutination kit of *Nosema bombycis*, *Nosema* strains viz., M11 and M12 indicating that Lamerin microsporidia is serologically different from them. The polyclonal antibody raised against the spore of Lamerin microsporidia did not cause agglutination of spores *N. bombycis*, confirming that the Lamerin microsporidia is serologically different from the standard strain *N. bombycis*.
- ❖ The Lamerin microsporidian spores germinated to the extent of 100% on incubation for 30 min. in KOH of 1% concentration and its neutralization. It

differed from *N. bombycis* spores which hatched at KOH concentration of 0.1-1%.

- ❖ Investigations on the portals of infection indicate that the Lamerin microsporidia infects through oral portal and transovum portals. The cutaneous portal did not cause infection at all. Infection through oral portal caused only  $9.8 \pm 0.83$  and  $3.2 \pm 0.83\%$  mortality in larval and pupal stages. It is also observed that  $63.93 \pm 3.24\%$  progeny from the laying of the infected moths were infected. The transovum portal caused  $11.80 \pm 0.84$  and  $5.20 \pm 0.84\%$  mortality in larval and pupal stages.
- ❖ Lamerin microsporidia infects Lamerin, PM and CSR2. breeds of silkworm. confirming that the microsporidian infection is not confined to Lamerin breed alone but also infects other multivoltine and bivoltine breeds. However the infectivity of Lamerin microsporidia is much lower than the *N. bombycis*. It ranged from  $41.20 \pm 3.49$  -  $48.00 \pm 1.58\%$  as against  $87.55 \pm 4.19$  -  $91.69 \pm 3.33\%$  by *N. bombycis*.
- ❖ Investigations on the mode of transmission of Lamerin microsporidia in three-silkworm breeds viz., Lamerin, Pure Mysore and CSR2 indicate that the infection is transmitted to the progeny possibly by transovarial transmission. Lamerin microsporidia healthy female moth paired with infected male moth lay eggs whose progeny were free of infection. Lamerin microsporidian infected female moth paired with healthy male moth lay eggs whose progeny carry infection. This mean that the male moth though carry the infection do not transmit infection to the progeny. The infected female moth always transmits infection to the progeny.
- ❖ The rate of transmission of Lamerin microsporidian infection in Lamerin, Pure Mysore and CSR2 breeds was comparatively lower than transmission rate by *N. bombycis*. The rate of Lamerin microsporidian transmission in three

breeds ranges from 56.80±3.92% - 64.00±6.78%. The percent transmission of infection of *N. bombycis* to progeny is 100 %.

- ❖ Apart from low infectivity and low rate of transmission, the Lamerin microsporidia spreads infection in healthy silkworm colony at low rate. The Lamerin microsporidian carriers 1-9 in number, introduced in a healthy silkworm colony of 100 larvae did not spread infection that lead to mortality in larval stage but infection was observed in moth stage.
- ❖ Lamerin microsporidia infects gut tissue, malphgian tubules, trachea, silk gland and gonads. Compared to *N. bombycis*, the rate of infection of Lamerin microsporidia was low. It takes nine days after *per os* inoculation for the first appearance of spores in the gut while in case of *N. bombycis* it was seen on the 6<sup>th</sup> day of post inoculation. The spore makes its appearance on the 13<sup>th</sup> day of post inoculation in trachea, gonad and haemolymph while in case of *N. bombycis*, the trachea, silk gland and gonads develop spores on the 7<sup>th</sup> day itself. The intensity of infection in different tissues by Lamerin microsporidian is also lower than the intensity of infection caused by *N. bombycis*.
- ❖ Lamerin microsporidia is less virulent than *N. bombycis*. The LC<sub>50</sub> dose of Lamerin microsporidia to 3<sup>rd</sup> instar Lamerin, Pure Mysore and CSR2 breeds is  $8.7 \times 10^9$ ,  $4.7 \times 10^8$  and  $2.8 \times 10^5$  spores/ml while for *N. bombycis* the LC<sub>50</sub> value for the same breeds is  $1.7 \times 10^6$ ,  $9.1 \times 10^5$  and  $1.6 \times 10^5$  spore / ml respectively. The Lamerin microsporidia is 5117, 516 and 1750 times less virulent than *N. bombycis* to Lamerin, Pure Mysore and CSR2 breeds of silkworm respectively.
- ❖ Different breeds of the silkworm respond differently to microsporidian infection (Lamerin microsporidian and *N. bombycis*). CSR2 was most susceptible to Lamerin microsporidia as well as *N. bombycis*. Lamerin breed is least susceptible to both Lamerin microsporidia and *N. bombycis*.

- ❖ Lamerin microsporidian causes infection and lowers the percent survival of the silkworm breeds screened. The survival percent due to Lamerin microsporidian infection was lowest in breed CSR2 (80.40%) followed by CSR19 (82.20%), CSR18 (82.60%), NB4D2 (84.40%), Nistari (85.00%) and PM (85.80%). However the maximum survival of 89.20% was recorded with Lamerin breed as against the healthy host survival of 96.20%. Although high survival is observed, 40.00 to 49.5% of the moths emerged were infected. The single cocoon weight, shell weight and percent silk content were also lowered by the Lamerin microsporidian infection.
- ❖ A comparative study of Lamerin microsporidian and *Nosema bombycis* infections in above stated breeds indicates that the *N. bombycis* is comparatively more virulent than Lamerin microsporidia. However the survival percent (55.60%) of Lamerin breed against *N. bombycis* is comparatively higher than the other breeds against *N. bombycis*. In CSR2, CSR18, CSR19, NB4D2, Pure Mysore and Nistari the survival percent were 25.60%, 21.80%, 24.40%, 27.40%, 42.80% and 36.40% respectively. All moths (100%) emerged from different breeds were found infected with *N. bombycis*.
- ❖ Among physical disinfectants screened against Lamerin microsporidia, the Ultraviolet rays (1200sec.). Hot water (100°C) and Hot air (100°C) were observed to be sporicidal against Lamerin microsporidia.
- ❖ Among the chemical disinfectants Chlorine dioxide (400ppm for 20min), bleaching powder of 30% chlorine (1% for 10min), Formalin 37% formaldehyde (1% 20min) and phenol (9% for 10min) were effective against Lamerin microsporidia. Potassium permanganate was not effective at tested concentrations.
- ❖ Thermal treatment of eggs infected with Lamerin microsporidia reduced the mortality in progeny due to the Lamerin microsporidian infection. In the infected control laying the percent of hatching is 84.44% while in the

treatments involving temperature of 40 and 45°C, it ranged from 90-65 - 94.78%. The percent of dead larvae due to the microsporidian infection in different treatments was lower and it ranged from 66.86–100%. The percent of infection at moth stage were also lowered from 45.26% to 10.70 - 12.08%.

- ❖ Chemotherapy of microsporidiosis caused by Lamerin microsporidia was attempted with drugs/chemicals and plant extracts. Among the drugs, the benzimidazole derivatives, Albendazole at 1.00% and Mebendazole at 0.25 – 1.00% concentrations are effective in reduction of larval mortality due to microsporidiosis caused by Lamerin microsporidia to an extent of 100%. Among the quinine derivatives Saproquine at 0.25 - 1.00% concentration is effective in reduction of larval mortality to an extent of 100% in Lamerin and CSR2 breeds. However the moths were found infected and the percent of infected moths were significantly lower in all treatments.
- ❖ Among the botanicals screened the aqueous extracts at 1-3% concentration of seeds of *A. copticum* (Ajowan) of family *Umbelliferae*, and *C. paradisi* (Grape seed) of family *Rutaceae*, were effective in reduction of mortality caused by Lamerin microsporidia to an extent of 100%. The percent of moth infected was also reduced. The percent moth infected in the inoculated control was 63.82% in Lamerin and 62.86% in CSR2 silkworm breeds. It was reduced to 13.51 - 18.65% in Lamerin and 10.37 - 12.88% in CSR2 breeds by extracts of seeds of *Ammi copticum*. The extracts of seeds of *Citrus paradisi* reduced the percent moths infected to 15.58±1.47% - 21.02±1.62% in Lamerin and to 10.07 ±1.01% - 12.96±1.14% in CSR2 breed.

## CONCLUSIONS

The microsporidian isolate from Lamerin breed of the silkworm *B. mori*, L. is a typical microsporidia and it differs from most common microsporidia (*N. bombycis*) infecting silkworm by its morphological, biochemical, ultrastructure and serological features. It infects by oral portal. The infection is passed to the progeny by infected female and possibly by transovarial means. The rate of

transmission is significantly lower than that of *N. bombycis*. The Lamerin microsporidia is less virulent than *N. bombycis* and more so to the Lamerin breed. Lamerin breed is also more tolerant than other breeds. The physical agents such as hot water, hot air and ultraviolet rays and chemical disinfectants such as Chlorine dioxide, bleaching powder and formalin commonly used in sericulture were also effective against the spores of Lamerin microsporidia. Thermotherapy of Lamerin microsporidia infected eggs is possible with temperature treatment of eggs at 45°C for 10min. Chemotherapy of Lamerin microsporidian infected larvae is also possible with *per os* treatment of derivatives of benzimidazole viz., Albenmidazole and Mebendazole and extracts from seeds of *A. copticum* and *C. paradesi*.