



Studies on synergistic effects of different chemicals on nuclear polyhedrosis (grasserie disease) of silkworm, *Bombyx mori* Linnaeus

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Abstract

Larvae of multivoltine breed silk worm *Bombyx mori* L. (Nistari) was studied in both favorable and unfavorable rearing seasons after Grasserie disease caused by *Bombyx mori* Nuclearpolyherosis Virus (*BmNPV*) using various disinfectants. Chemical treatments using various complex mixtures were administered to minimize the extent of damage, which in turn may help the stakeholders to step ahead on the progression of sustainable sericulture by avoiding huge crop loss.

Keywords: *Bombyx mori* L, Nistari, Multivoltine breed, Nuclearpolyherosis Virus (*BmNPV*), Favorable rearing season

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1. Introduction

'Prevention is better than the cure' is highly applicable to the integrated disease management. It means that one should go about actively preventing diseases before they occur and it is only when preventive precautions are in force, we can hope to effectively manage the occurrence and spread of disease. Dependence on treatment after the disease appearance will always entail losses. In short the fundamental basis of silkworm disease management is the implementation of the policy of prevention first. Under this policy disinfection to eliminate risk of disease transmission and increasing the vigor of the silkworm to enhance the resistance to diseases are given due weightage. Sanitation before, during or even after each rearing should be carried out effectively and strictly. One has to possess knowledge to intensify the feeding and management to comply with the physiological requirements of the silkworm for raising healthy silkworms. The effect of various diseases on protein metabolism of silkworm larvae has been studied by different investigators; however, the possible effect of the grasserie infection on protein metabolism in various tissues of pre-spinning silkworm larvae has not been worked out. It will be worthwhile to make an attempt toward understanding the alterations in protein fractions of pre-spinning silkworm larvae during grasserie.

Several studies have reported the induction of insect viral diseases upon exposure to various chemicals like formalin, hydrogen peroxide, potassium nitrate, hydroxyl amine, etc. (Aruga, 1963; and Yamafuji and Yoshihara, 1950). Leaves smeared with formalin at 8% and fed to silkworms induce grasserie. Ishimori and

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Muto (1951) opined that *BmNPV* gets induced by sudden decrease in catalytic activity in the body fluid of larvae and formation of viral protein due to protein exposure to hydrogen peroxide treatment. However, a pertinent question often arises in every sericulturist that if the parent generation suffers from *BmNPV* infection, would the offspring carry the same through transovarial transmission or not. In this context disinfection plays a vital role in sericulture during rearing of silkworm. The success in sericultural production depends much on the provision of 'Germ free environment' and the credit goes to a large extent to disinfection and hygiene. Disinfection is the major factor which determines the success of rearing and cocoon production.

Disinfection is the destruction and extermination of disease causing germs. Proper disinfection of silkworm disease is one of the key factors in determining the success of sericulture (Anonymous, 1990). The aim of disinfection and hygiene is to create a pathogen free environment for cocoon production. In tropical conditions, out of five to six crops in a year, the sericulturists experience two to three crop failures or poor yields due to silkworm diseases which are very high during rainy and dry months. Kotikal et al. (1989) studied the role of disinfection of silkworm management and reported that the attack of various diseases to the silkworm leads to an annual crop loss to 30-40%, particularly in India. The problem of diseases can be largely overcome by concentrating on prevention rather than trying to control them after their outbreak (Baig and Kumar, 1987). The important prophylactic measures followed are disinfection of rearing house and the rearing appliances in addition to incubation care, feeding the larvae only with good quality mulberry leaves, management of young and late age to raise robust stock to increase their vigor and level of disease resistance (Noamani et al., 1989).

The physical methods of disinfection include simple and effective physical treatment for prevention and control of silkworm diseases (Jolly, 1986). Disinfection by exploiting the natural sunlight can be considered as a supplementary measure for pathogen destruction. The germicidal effect of sunlight is derived from UV radiation which kills the pathogens (Chaun and Chaung, 1988). Subbaiah et al. (1990) reported that exposure of contaminated rearing appliances to sunlight resulted in lowering the diseases, indicating its efficacy in inactivating the pathogen. The chemicals affect the metabolism of pathogens through oxidation or reduction of tissues (Anonymous, 1975). Disinfection of rearing house is done three to ten days before starting the rearing operation. The effectiveness of chemical disinfectant depends upon three factors namely the concentration, the duration and ambient temperature (Benchamin and Nagaraj, 1987). Fumigation is a method of disinfection involving use of the disinfecting chemicals in its volatile or gaseous form. The rooms to be fumigated should be necessarily made air tight, as the effect of fumigation depends on the swift diffusion of the vapors into all parts of air space (Jolly, 1986).

Veeranna (1999) reported that of the several means for prevention, control and management of silkworm diseases, disinfection of rearing houses and equipment using 2% formalin at the rate of 800 ml/ 10 square meter area is important. Formalin is the most widely used disinfectant in sericulture which is effective as a spray and fumigation. It is also used as a medium for wash (Baig and Kumar, 1987). The commercial formalin with strength of 35-38% formaldehyde has been used for disinfection of rearing rooms prior to brushing (rearing) as of its germicidal effect and it is also used as a gaseous sterilizing agent, but the process suffers from disadvantages including stability of stock solution, toxicity (Threshold limit value 2 ppm commercial formalin of 36%) and it is used to generate and evenly distribute the gas (Newson and Mathews, 1981). Being aqueous solution of formaldehyde, it has a strong reduction effect and the germicidal action caused by depriving the oxygen of the pathogens. The disinfecting efficiency of this chemical increases as the temperature rises above 24 °C (Jolly, 1986). The germicidal effect is mainly on nuclear polyhedrosis virus causing grasserie. However, its effect is found to be weak on cytoplasmic polyhedrosis virus (Chaun and Chaung, 1988). Several workers have conducted experiments on this aspect and the results are highly contradictory. In these experiments even though virus infections are confirmed in the parent generations but the virus disease was not observed in their offspring reared in aseptic conditions. Further studies conducted by Matsubara (2001) to find out the factors responsible for inducing viral diseases in silkworms reared normally (without aseptic condition). If the rearing environment, egg shell, mulberry leaves or artificial diets are contaminated, the virus enters in the silkworm body through the pre-oral route and causes latent infection. From these results it was concluded that the polyhedral viruses are not transmitted to the next generation through the eggs, so, induction of virus diseases is due to infection and the inducing factors will only cause physiological disorders in silkworms. In physiologically disturbed conditions the virus can easily infiltrate into the silkworm body, infect and propagate ultimately causing a disease (Matsubara, 2001). Therefore, before developing any method to control grasserie, there is a need to understand factors responsible for the physiological disorders in silkworms. Nevertheless,

general precautionary measures emphasize to rear silkworms in optimum condition of temperature and humidity and supply of nutritious food in order to enhance the host's natural defense system against pathogens be strictly followed for protecting silkworms from grasserie (Deb et al. 2015).

Defense mechanism in silkworm against grasserie may be highlighted as prevention of diseases, and breeding of silkworm varieties with high degree of resistance are important aspects in commercial sericulture (Aizawa, 1959).

B. mori is reared in different geographical areas including temperate and tropical regions with different races. It is possible for variations in the biological and genetical features. Physical properties of the silk, softness and illustriousness of the silk fabrics made out of tropical multivoltine races are superior to temperate bivoltine races. However, races of tropical region have special biological characteristics of short growth phase ranging from embryonic to pupal stages. The present study emphasizes on the influence of environmental factors on Nistari (multivoltine) silkworm breed in West Bengal. The three major features of insects i.e. size, impermeability and rigidity of exoskeleton and their poikilothermy have importance in determining the physiological relationship with the environment. Success of insect performance depends on its ability to maintain a stable internal state within certain tolerable limits of temperature, osmotic pressure, pH and oxygen concentration (homeostasis) along with the pathogen load (Baig and Kumar, 1987). It has been further characterized that most of the insects including silkworm possess extensive defense against virus invasion and in this context it would be useful to examine events at molecular level for its application (Dutta and Ashwath, 2000).

2. Materials and methods

Sericulture is an agro-based cottage industry mainly based on mulberry plantation, silkworm rearing and reeling. It plays an important role in the upliftment of the rural people and minimizes the unemployment problem by providing engagement to the people. In this context disinfection plays a vital role in sericulture during rearing of silkworm. The success in sericultural production depends much on the provision of 'Germ Free Environment' and the credit goes to a large extent to disinfection and hygiene. Disinfection is the major factor which determines the success of rearing and cocoon production. Silkworm rearing during unfavorable season needs to do several measures to prevent pathogen by using disinfectants. The rearing room should be kept with a good hygienic condition because during unfavorable season silkworm rearing may be affected by many diseases. It causes less cocoon crop and poor yield also. Implementation of a scientific method of disinfection and hygiene is required for the development of sericulture industry. Therefore, the present study is an attempt to bring out the comprehensive account of the prospect of sericulture activities.

Trichodermin: *Trichoderma viridae* the potential antagonistic fungus which prevents the crops from diseases, viz., Root rots, wilts, brown rot, damping off, charcoal rot and other soil borne diseases in crops. *Trichoderma* is able to suppress more than 60 species of pathogens (*Pythium*, *Botritis*, *Phoma*, *Sclerotinia*, *Fusarium*, *Ascochyta*, *Alternaria* and others) on different plants (cucumbers, tomatoes, cabbages, peppers, various ornamentals, cereals and grain legume crops). It is used for controlling the diseases of sugarcane, pulses, oilseeds, cotton, vegetables, banana, coconut, oil palm, chillies, lime, coffee, tea, areca nut, rubber, flower crops, spices, etc.

The active components of bio-pesticides made on the base of this fungus-antagonist are their spores, mycelium and products of metabolism. This fungus secretes cellulase and chitinase enzymes which react with cell wall of the pathogenic fungi or bacteria and dissolve the same. *Trichoderma* utilize the protoplasm as a source of food and multiply its spores. By this method the spores of the pathogenic fungi are destroyed. In the process of development *Trichoderma* synthesizes a variety of antibiotics (gliotoxin, viridine, trichodermin and others). They destroy the cell walls of phytopathogenic fungi and produce biologically active substances, which stimulate plant growth and development. *Trichoderma* possess innate resistance to most agricultural chemicals, including fungicides, although individual strains differ in their resistance. The fungicidal activity makes *T. viride* useful as a biological control agent against plant pathogenic fungi. It has been shown to provide protection against such pathogens as *Rhizoctonia*, *Pythium* and even *Armillaria*. It is found naturally in soil and is effective as a seed dressing in the control of seed and soil-borne diseases including *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium* species. When it is applied at the same time on the seed, it colonizes on the seed surface and kills not only the pathogens present on the cuticle, but also provides protection against soil-borne pathogens.

Paraformaldehyde: Paraformaldehyde is the smallest polyoxymethylene, the polymerization product of formaldehyde with a typical degree of polymerization of 8-10 units. Paraformaldehyde commonly has a slight odor of formaldehyde due to decomposition. Paraformaldehyde is a polyacetal. Paraformaldehyde forms slowly in aqueous formaldehyde solutions as a white precipitate, especially if stored in the cold. Formalin actually contains very little monomeric formaldehyde; most of it forms short chains of polyformaldehyde. A small percent of methanol is often added as a stabilizer to limit the extent of polymerization. Paraformaldehyde can be depolymerized to formaldehyde gas by dry heating and to formaldehyde solution by water in the presence of a base or heat. The very pure formaldehyde solutions obtained in this way are used as a fixative for microscopy and histology. The resulting formaldehyde gas from dry heating paraformaldehyde is flammable. Once paraformaldehyde is depolymerized, the resulting formaldehyde may be used as a fumigant, disinfectant, fungicide and fixative.

1, 3 Dibromo 5, 5 Dimethylhydantoin (DBDMH): DBDMH is an organic compound derived from the heterocycle called dimethylhydantoin. This white crystalline compound with a slight bromine odor is widely used as a disinfectant used for drinking water purification, recreational water treatment, as a bleaching agent in pulp and paper mills, and for treating industrial/commercial water cooling systems. Its action does not involve the use of hypochlorous acid. DBDMH is a source of bromine, which is equivalent to hypobromous acid (HOBr). $\text{Br}_2\text{X} + 2\text{H}_2\text{O} \rightarrow 2\text{HOBr} + \text{H}_2\text{X}$, (Where H_2X is 5, 5-dimethylhydantoin). With a pKa of 8.6, hypobromous acid partially dissociates in water: $\text{HOBr} \rightleftharpoons \text{H}^+ + \text{BrO}^-$. Hypobromous acid serves as a source of "Br⁺," which produces bromide ions in the process of disinfection: $\text{HOBr} + \text{live pathogens} \rightarrow \text{Br}^- + \text{dead pathogens}$. The resulting bromide ions can then undergo oxidation to hypobromous acid in the presence of an oxidizer of sufficient strength, e.g., ozone, hypochlorous acid, potassium monopersulfate. This reoxidation process is commonly called "activation" of the bromide ion: $\text{Br}^- + \text{HOCl} \rightarrow \text{HOBr} + \text{Cl}^-$. It is an external broad-spectrum disinfectant, bactericidal, algacidal deodorant. It can be used in the infection of silkworm breeding, livestock and fish breeding, etc. It also can be used in the anti-shrinking finishing for wool, bleaching in textile industry, as algacide for industrial recirculating water, as rubber chlorinating agent, etc. It has stable and high-efficiency performances, no any bad effects on the human body.

Bleaching powder: Bleaching powder is a white amorphous powder, with a pungent smell of chlorine. For effective disinfection, a high grade of bleaching powder with an active chlorine content of 30% must be used. Bleaching powder solution (5%) can be prepared by dissolving 50g of bleaching powder in 1l of water. This solution contains 1.6% active chlorine. For room disinfection one liter solution is required to disinfect 2.5 sq. m areas. The available chlorine in commercial bleaching is around 25-30% and is not completely soluble in water and hence only the supernatant is used for spraying.

Lime: Three rearing per year were conducted with multivoltine Nistari during June-July 2017-20, July-August 2017-20 and August-September 2017-20 at Silkworm Pathology Laboratory, Central Sericultural Research and Training Institute, Berhampore, West Bengal, India, to know the efficacy of different bed disinfectants. At the outset of rearing, the rearing room and appliances like *Chandraki*, etc. were washed thoroughly with sprinkle of water. Then the appliances were kept in the rearing room and disinfected the rearing room and appliances with 5% bleaching powder solution as per recommendation of the Institute.

Application of disinfectants: Five sets of treatment, each with three replications were kept in the rearing room and larvae were brushed normally on the paraffin sheet and fresh mulberry chopped leaves were fed to the silkworms and allowed the silkworm for development. Hatching% were counted and recorded. Temperature and R.H% were also recorded. The IIIrd instar larvae were counted after undergoing their second molt and 100 larvae were kept for each treatment and replication. Then larvae kept in five trays were dusted with different bed disinfectants whose compositions are given below:

Dusting-1: Trichodermin, Paraformaldehyde and Lime (30: 20: 950)

Dusting-2: Paraformaldehyde and Lime (20: 980)

Dusting-3: 1, 3 Dibromo 5, 5 Dimethylhydantoin (DBDMH), Paraformaldehyde and Lime (30: 20: 950)

Method of application: Chemicals and botanicals are screened as inducer was dusted with selective dose to the larvae after resuming from 4th molt (1st day '0' hr). Dusting was done 3-4 g/ sq. ft. of bed area on silkworm body. Dusting was done on silkworm larvae after each moult 30-45 min before the resumption of feed. One additional dusting was done on the 4th day of Vth instar after bed cleaning. On the appearance of disease

symptom, dusting frequency increased every day. Bed disinfectants were dusted once 30 minutes after resume from each molt and an additional dusting was done on 5th day of the Vth instar after bed cleaning. For dusting muslin cloth was used. During the course of rearing, disease wise larval mortality were recorded. Mortality was also recorded during harvesting of cocoons. In this study, data pertaining to Effective Rearing Rate (ERR) %, Single Cocoon weight, Single Shell weight and Shell (%) were recorded.

Data collection: Larval mortality percentage (%) was recorded till the formation of cocoon. Mature larval weight (wt.), Cocoon wt., Shell wt., Effective Rearing Rate (ERR) % was recorded. The data was analyzed statistically to verify the result.

3. Results and discussion

Preventive measures: Following the methodology stated earlier, the chemical treatment was conducted to study the effect of different chemicals to control grasserie.

Larval mortality: Range of larval mortality was significant during all unfavorable seasons. In all three rearings, in the *BmNPV*-challenged larvae, Dusting 1 performed better. In the 1st rearing, average relative humidity was observed 79.28% coinciding with an average temperature of 28.84 °C. In the 2nd rearing, average humidity was 84.67% coinciding with an average temperature of 28.62 °C. In the 3rd rearing average relative humidity was 80.13% while average temperature of 31.59 °C was observed. Multiplication of pathogen depends upon the age of silkworm and time dependent and other indirect factors (Solter et al., 1990). Host and parasite share a sub-optimal temperature for optimal development, the parasite appears to be more sensitive to lower temperature than host and temperature is one of the important factors that determine the multiplication of pathogens (Madanamohan et al., 2006). The present findings corroborate the findings of Ghosh and Saha (1995). In general, the mortality of insect is directly related to the size of the pathogen received. Weiser (1976) stated that time taken for establishment of the pathogen depending on environmental factors. Multiplications of pathogens depend upon the age of silkworm, time and other indirect factors (Solter et al., 1990). Optimum temperature is most favorable for pathogens multiplications and high temperature or equal to the insect's thermal threshold (15-27 °C) has a little influence on the development of disease. It may be stated that besides temperature, relative humidity is an important environmental factor affecting profoundly on growth and development of mulberry silkworm during infection.

Effective Rate of Rearing (ERR %): ERR was recorded highest during August-September rearing season, 2017-20, compared to the first and second seasons as the season was better (Tables 5 and 6). Silkworm larvae treated with Dusting 2 had highest ERR% under normal conditions while those treated with Dusting 1 showed high ERR% when challenged with *BmNPV*.

Mature larval weight (g): In the first rearing, the normal control, mature larval weight was found to be higher in Dusting 1 (1.33 g) followed by Dusting 1 (1.21 g), Dusting 2 (1.14 g), and Dusting 3 (0.96 g). In the *BmNPV* challenged larvae, the weight was maximum in Dusting 1(1.15g), followed by Dusting 2 (1.10g) and Dusting 3(0.87 g) (Tables 1 and 2). In the second rearing, the normal control, mature larval weight was found to be higher in Dusting 1 (2.01 g), Dusting 3 (1.76 g) and Dusting 2 (1.75 g) (Tables 3 and 4). In the *BmNPV* challenged trays, the weight was maximum in Dusting 1 (1.73 g), followed by Dusting 2 and Dusting 3 (1.66 g).

Table 1: Rearing- I: Economic parameters before challenge with *BmNPV* during June-July, 2017-20

Treatment	V th Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.207 ± 0.019	44.000 ± 0.408	1.264 ± 0.001	0.219 ± 0.000	17.139 ± 0.008
Dusting 2 (D2)	1.140 ± 0.025	41.667 ± 0.624	1.227 ± 0.001	0.218 ± 0.001	16.637 ± 0.006
Dusting 3 (D3)	0.963 ± 0.006	43.333 ± 0.624	1.265 ± 0.002	0.213 ± 0.001	16.804 ± 0.006
CD (Critical Difference)	0.110**	2.481**	0.005**	0.002**	0.047**
CV% (Critical Variance)	3.627	2.035	0.159	0.361	0.104

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.150 ± 0.004	38.000 ± 0.408	1.248 ± 0.001	0.209 ± 0.001	16.546 ± 0.013
Dusting 2 (D2)	1.100 ± 0.004	31.333 ± 0.624	1.244 ± 0.002	0.202 ± 0.001	16.023 ± 0.010
Dusting 3 (D3)	0.867 ± 0.015	29.333 ± 0.471	1.231 ± 0.001	0.201 ± 0.001	16.097 ± 0.006
CD (Critical Difference)	0.069 **	0.964 **	0.007 **	0.006 **	0.059 **
CV% (Critical Variance)	2.470	7.921	0.223	1.017	0.136

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	2.007 ± 0.010	53.667 ± 0.624	0.905 ± 0.002	0.103 ± 0.001	10.939 ± 0.026
Dusting 2 (D2)	1.753 ± 0.010	47.000 ± 0.816	0.857 ± 0.002	0.099 ± 0.002	9.988 ± 0.007
Dusting 3 (D3)	1.757 ± 0.008	40.333 ± 0.624	0.746 ± 0.001	0.096 ± 0.000	10.977 ± 0.004
CD (Critical Difference)	0.082**	3.929**	0.025**	0.004**	0.085**
CV% (Critical Variance)	1.652	2.969	1.048	1.610	0.299

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.727 ± 0.006	49.000 ± 0.408	0.848 ± 0.001	0.093 ± 0.001	10.827 ± 0.008
Dusting 2 (D2)	1.657 ± 0.009	35.000 ± 0.408	0.858 ± 0.005	0.090 ± 0.001	9.987 ± 0.008
Dusting 3 (D3)	1.661 ± 0.012	36.000 ± 0.624	0.739 ± 0.001	0.086 ± 0.001	10.670 ± 0.008
CD (Critical Difference)	0.051**	4.452**	0.016**	0.005**	0.051**
CV% (Critical Variance)	1.135	4.053	0.774	2.178	0.182

In the third rearing, the normal control, mature larval weight was found to be higher in Dusting 1 (1.77 g), Dusting 3 and Dusting 2 (1.65 g) (Tables 5 and 6). In the *BmNPV* challenged trays, the weight was maximum in Dusting 1 (1.67g), followed by Dusting 3 (1.65g), Dusting 2 (1.62 g). The extent of weight loss, however, cannot be directly linked to that of the lower food intake and conversion efficiency alone, because the weight reduction was alone caused by the parasite exploiting the nutritional resource (Ponnuvel et al., 1997). Under the influence of pathogen, the host metabolism being diverted from pyruvate to oxaloacetate oriented reactions might have something to do with replenishment of hosts' glycogen reserve and the host metabolism is geared towards meeting the requirements of the growing pathogens (Sharan et al., 1998).

Table 5: Rearing - III: Economic parameters before challenge with *BmNPV* during Aug- Sep, 2017-20.

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.770	93.000	0.752	0.093	12.420
Dusting 2 (D2)	1.653	92.667	0.706	0.085	12.005
Dusting 3 (D3)	1.660	91.667	0.730	0.085	11.876
CD (Critical Difference)	0.033**	NS	0.014**	0.005**	0.200**
CV% (Critical Variance)	0.729	0.935	0.737	1.911	0.617

Table 6: Rearing - III: Economic parameters after challenge with *BmNPV* during Aug- Sep, 2017-20

Treatment	Vth Instar Larval Weight (g)	Effective Rearing Rate (%)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell (%)
Dusting 1 (D1)	1.670	82.407	0.726	0.087	12.344
Dusting 2 (D2)	1.617	75.667	0.668	0.082	11.993
Dusting 3 (D3)	1.647	77.000	0.710	0.083	11.573
CD (Critical Difference)	0.02**	3.976**	0.028**	NS	0.085**
CV% (Critical Variance)	0.463	1.912	1.510	1.886	0.267

Single Shell Weight (SSW): Single Shell Weight is the weight (in grams) of a single cocoon shell. SSW in the 1st rearing was seen to be in the range of 0.20 to 0.22 g (Tables 1 and 2). In the second rearing SSW was in the range of 0.08 to 0.09 g and in the 3rd rearing, SSW ranged from 0.09 g to 0.11 g.

Single Cocoon Weight (SCW): Single Cocoon Weight is the weight (in grams) of a single cocoon, i.e., the combined weight of one shell and the pupa inside. SCW in the 1st rearing ranged from 1.23 g to 1.35 g. In the 2nd rearing, it ranged from 0.71 g to 0.86 g (Tables 3 and 4) and in the 3rd rearing it ranged from 0.71 g to 0.78 g.

4. Conclusion

Preventive measure of grasserie infected silkworms indicates interesting findings. High temperature and relative humidity act as stimulatory factors for multiplication of pathogens resulting in higher mortality in season-2 (S2). Solter *et al.* (1990) support the present findings. Multiplication of pathogen depends upon the age of silkworm and time dependent and other indirect factors. Host and parasite share a suboptimal temperature for optimal development, the parasite appears to be more sensitive to lower temperature than host and temperature is one of the important factors that determine the multiplication of pathogens (Madanamohan *et al.*, 2006). The present findings corroborate the findings of Ghosh and Saha (1995). The prevailing temperatures in S2 were favorable for appearance of diseases. In general, the mortality of insect is directly related to the size of the pathogen received. Weiser (1976) stated that time taken for establishment of the pathogen depending on environmental factors. Multiplication of pathogens depends upon the age of silkworm, time and other indirect factors. Optimum temperature is favorable for pathogen multiplications. It may be stated that besides temperature, relative humidity is an important environmental factor affecting on growth and development of mulberry silkworm during infection, and silkworm can tolerate minor variation of temperature as prevailed in season-1 but small variation of relative humidity disfavors the development of silkworm and favors the multiplication of pathogens as prevailed in season-2 (Chakrabarty and Manna, 2008). Effective Rearing Rate (ERR) was

found significant ($p < 0.1$) during second unfavorable seasons. ERR was recorded significantly higher during first season compared to second season. The extent of weight loss, however, cannot be directly linked to that of the lower food intake and of conversion efficiency alone, because the weight reduction was alone caused by the parasite exploiting the nutritional resource (Ponnuvel et al., 1997). Under the influence of pathogen, the host metabolism being diverted from pyruvate to oxaloacetate oriented reactions might have something to do with replenishment of host's glycogen reserve and the host metabolism is geared towards meeting the requirements of the growing pathogens (Sharan et al., 1998). There was positive correlation of ingestion of food with larval weight, cocoon weight and shell weight (Magadum et al., 1996). Diseased silkworms spun flimsy cocoons, which are unfit for reeling (Geethabai and Mahadevappa, 1995). In addition to retardation of growth, the economic parameters were also adversely affected by infection lending credence to the fact that inhibition of normal growth /development results in poor cocoon and silk quality (Krishnan et al., 1998).

During normal silkworm rearing conditions, Dusting-1 performed better when the larvae were challenged with *BmNPV* and it increased ERR ~ 3.12% (56.47%). Dusting 1 contains *Trichoderma* (biofungicide), paraformaldehyde and lime (30: 20: 950) which have better efficiency in case of challenge with *BmNPV*. Dusting 2 contains Paraformaldehyde and Lime (20: 980) which have better efficiency in case of challenge with *BmNPV*. However, Dusting-1 performed comparatively better when the larvae were challenged with *BmNPV* as it increased ERR 56.47% over the Dusting 2. Dusting 1 contains Trichodermin, Paraformaldehyde and Lime (30: 20: 950) which have better efficiency in case of challenge with *BmNPV* over Dusting-2 which contains Paraformaldehyde and Lime (20: 980) due to the synergistic effect of additional component of Trichodermin, bio fungicide prepared from *Trichoderma viridae* (1%).

Full disinfection: The laboratory rearing conducted during unfavorable season shows that 52.1% total mortality and 15.66% in Dusting 3 and also ERR No. was 4790 and 8434 respectively in control and Labex dusted lot. Regarding mature larval weight the result was 17.812 and 18.065 g respectively.

Partial disinfection: In the case of partial disinfection, total mortality percentage was 77.67 and 21.33 in control and Dusting 1, dusted lot respectively. Regarding ERR by number the result was 2233 and 7867 and ERR% was 12.809 and 13.570 respectively in control and Dusting 1. In partial disinfected room three other disinfectants were used for the same study. From the result it is very much clear that full disinfection, partial disinfection and without disinfection the silkworm rearing result is very much varied. When the disease wise mortality was considered the highest mortality was recorded due to grasserie (34.33%) in lot without any disinfection. But at the same time mortality due to grasserie was 5.5% and 9.33% in fully disinfected and partially disinfected lot respectively. The same trend was also reflected in total mortality that is 77.67% (without any disinfectant), 21.33% (partial disinfection) and 15.66% (full disinfection). Regarding ERR number the highest is in fully disinfected lot (8434) compared to partial disinfection (4790). When cocoon character was considered, the influence of full disinfection is positively reflected. In the case of silk ratio fully disinfected lot showed better (13.672%) compared to other treatments (partially disinfected-13.570% and without disinfection-12.890%). Similarly, cocoon per kg also showed influence of disinfection. But the mature larval weight did not show any significant difference in the three categories. From the field survey data it was evident that the framers are doing rearing beyond their capacity that is one farmer brushed nearly 500 dfls but he has only 50 trays. Because of this in the later stages of rearing larval mortality is high which finally leads to high crop loss. Here the crop loss range is 25-50% and in one case it is 100%.

However, Dusting-1 performed comparatively better when the larvae were challenged with *BmNPV* as it increased ERR 56.47%. It has been known that bed disinfectant Bleaching powder and lime has a definite role in preventing the diseases by inactivating the pathogens but Dusting 1 contains Trichodermin (bio-fungicide), Paraformaldehyde and Lime (30: 20: 950) which have better efficiency in case of challenge with *BmNPV* over Dusting-2 which contains Paraformaldehyde and Lime (20: 980) and Labex due to the synergistic effect of additional component of Trichodermin, bio fungicide prepared from *Trichoderma viridae* (1%). The farmers are aware of various room disinfectants and bed disinfectants particularly Labex and formalin. In the case of room disinfection most of the farmers used 2% formalin solution even though the recommendation in 2% formalin and 0.5% slaked lime. This may be one of the reasons of high incidence of grasserie and gattine disease which are basically due to some viral pathogens. Data generated through intensive and extensive silkworm disease survey is the fountain head of all practical accomplishment in the field related to disease management. On the basis of survey result many practical technologies can be developed with primary emphasis being given to prevention of occurrence of disease. Strategies for effective management of disease outbreak in silkworm

rearing envisage through the extensive disease survey early diagnosis of disease and suggestion of suitable prophylactic measures to curb the losses encountered due to disease.

Disinfection forms an integral part of healthy and successful silkworm rearing. It aims a total destruction of pathogens. Several diseases caused by bacteria, viruses, fungi and protozoa affect silkworm rearing. These pathogens released by diseased silkworm easily accumulate and spread in the rearing environment through different routes. They are not easily destroyed and can persist/survive for long periods under congenial conditions. The spores of the pathogens, especially those of fungi are light in weight and can easily be drifted by air current resulting in easy spread of the disease. There are no curative methods for any of the silkworm diseases and prevention is the best option. This is achieved by adoption of proper and effective methods of disinfection and stepwise maintenance of hygiene during rearing. To realize the benefit of the disinfection to the full extent, attempt should be made to have synchronised disinfection (mass) and rearing at unitary level to promote sustainable sericulture.

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