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A Practical Technology for Diagnosis and Management of Diseases in Silkworm Rearing

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A practical technology for silkworm disease management at farmer's level was suggested and test verified for its efficacy and adaptability. The technology consisted of disinfection of rearing house and appliances, early disease diagnosis, personal and rearing hygiene and silkworm body and rearing seat disinfection. Besides, the supportive techniques associated in disease management are cited. The validation trials of the technology involving 845 farmers crops with 149,530 disease free layings (dfls) of CSR bivoltine hybrids confirmed the effectiveness of the technology which resulted an average yield of 68.98 kg cocoons/100 dfls.

Key words : Diseases, Immunodiagnosis, Disinfectants, Disinfection, Practical technology.

Introduction

The impact of diseases in silkworm, *Bombyx mori* L., rearing on silk cocoon production is significant. An annual loss of 20% of cocoon production is reported in India due to diseases (Chitra *et al.*, 1975). The diseases such as nuclear polyhedrosis, flacherie and pebrine cause cocoon crop loss during the early and later part of final instar silkworms with the initiation of infection during 1st to 3rd instars. It is well established that the cause of diseases is pathogens and the disease management practices prior to beginning of rearing and in early and later instar rearing lead to successful cocoon crop, irrespective of breed and season. Persistency of pathogen in the silkworm rearing environment, atypical symptoms of disease in early stages of infection and an efficient mode of dispersal of pathogens in silkworm colony is the primary constraints in diseases management. The environmental and nutritional factors such as temperature, humidity and quality of mulberry also influence the rate of development of diseases in silkworms.

The authors at CSR&TI., Mysore have developed specific methods/measures to overcome the constraints in silkworm rearing and formulated an integrated practical technology for disease diagnosis and management. The present study reports the results of validation trial of the technology conducted at farmer's level and discusses its adoption in sericulture.

Materials and Methods

The technology was validated during a period of four years (1997 - 2001) in different sericultural states *viz.*, Karnataka, Andhra Pradesh and Tamil Nadu in India. The technology was adopted in silkworm rearings covering. The technology was adopted in silkworm rearing covering 845 farmer's crops and 149,530 dfls. Silkworms of CSR2 x CSR4/CSR5 bivoltine hybrids were reared on mulberry shoots of V1, S36 or K2 mulberry varieties. The practical technology for diagnosis and management of diseases had the following components: 1) elimination of pathogen from silkworm rearing environment, 2) exclusion of pathogen entry into rearing house, 3) diagnosis for diseases and health check in early instar, and 4) prevention of spread of diseases in the rearing bed.

Elimination of pathogen from silkworm rearing environment

The component aims at elimination of pathogen from silkworm rearing environment, was implemented prior to the beginning of the rearing and it consists of disinfection of rearing house, its surroundings and appliances by chem-

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ical disinfectants. The disinfectants employed were stabilised by Chlorine dioxide of 500 ppm (2.5% Sanitech) prepared in 0.5% slaked lime or Bleaching powder (2% or 0.6% Cl_2) prepared in 0.3% slaked lime or formalin (2%) in water. The disinfectant was sprayed to silkworm rearing environment (rearing house and appliances etc.), its surrounding at the rate of 1.5 litres/sq meters of floor area. The passage to and the area in front of rearing house were disinfected by dusting 5% bleaching powder in slaked lime once in three days. The disinfectant was dusted at the rate of 200 g/sq. meters. The process of disinfection was carried out during a period of 5 - 6 days as per the following schedule and activities.

Exclusion of pathogen entry into rearing house

The exclusion of pathogen entry into the silkworm rearing house during the rearing was achieved by practicing personal and rearing hygiene as follows.

Personal hygiene: The hands were disinfected by dipping them in a basin containing 2.5% Sanitech (500 ppm Chlorine dioxide) in 0.5% slaked lime disinfectant solution. The feet were disinfected by washing them in well containing the above disinfectant solution at the entry of the rearing house. The hands were disinfected at the time of entry into rearing house, after screening the rearing bed for diseased larvae, bed cleaning and before feeding as well as while coming out of rearing house.

Rearing hygiene: The silkworm rearing bed was screened visually for disease larvae based on symptoms and picked using chopsticks/forceps. The diseased larvae were disinfected by disposing them into 5% bleaching powder in slaked lime and burring and/or burning them at later period. The rearing waste was disposed into waste pit and sprinkled 5% bleaching powder in slaked lime.

Diagnosis for diseases and health check in early instar

Early instar (Chawki) silkworms were reared in Chawki Rearing Centers (CRC) following standard method aimed at healthy and robust growth till the beginning of 3rd instar and were subjected to disease diagnosis to ascertain the health status of the worms. This was carried out in three steps.

Behavioral observations: Silkworms exhibiting abnormal behavioral changes such as inactiveness, under sized and unmoulted were collected and considered as potential pathogen carriers. These were subjected to microscopic examination and immunodiagnosis.

Microscopic examination: The under sized and unmoulted larvae were subjected to microscopic examination. The haemolymph was examined for *Bombyx mori* nuclear polyhedrosis virus (BmNPV) polyhedra, pathogenic bacteria, *Serratia marcescens* and Mycelial bodies of fungus. The larvae were also homogenized in sterile distilled water and examined for pathogenic bacteria, *Streptococcus, Staphylococcus* sp. and Microsporidian spores.

Immunodiagnosis: The under sized and unmoulted larval homogenate was examined for *Bombyx mori* infectious flacherie virus (BmIFV) by colloidal dye based dipstick immunoassay (Nataraju and Datta, 1999) and *Bombyx mori* densonucleosis virus (BmDNV) by immunodiffusion test.

Colloidal dye based dipstick immunoassay was conducted by incubating the 'dipstick' in 10% larval homogenate for 30 min. The stick was washed in distilled water, air dried and incubated in BmIFV dye reagent for 30 min. The stick was washed in distilled water and observed for blue coloured dot on the center of the nitrocellulose strip in positive sample.

Immunodiffusion test was conducted in agarose gel on a microslide. One percent agarose gel was prepared in buffer PBS (pH 7.2) and poured on to a microslide and cooled. A central and six peripheral wells were made using gel punch. The anti- BmDNV antibody was dispensed into central well and the peripheral wells were

Table 1. Activities and the schedule of disinfection

Day	Details of activity
After completion of previous rearing	• Collection and burning of diseased larvae, melted and flimsy cocoons. • Flaming the floss of rotary mountages and disinfection. • 1st disinfection of rearing house and appliances.
5 days before brushing	• Cleaning and washing of rearing house and appliances. • Sun drying of appliances.
4 days before brushing	• Additional disinfection of rearing house and appliances with 0.3% slaked lime solution.
3 days before brushing	• 2nd disinfection of rearing house and appliances.
2 days before brushing	• Dusting disinfectant in front and passage to rearing house. • Opening the windows of early instar rearing room/house for aeration.
1 day before brushing	• Preparation for brushing.

filled with the larval sample homogenate. The gel slide with samples were incubated at 37°C for 48 hrs and observed for precipitin line.

In addition to the samples under validation, an additional 50 samples from the general farmers rearing was collected for validation of diseases diagnosis method. This was necessitated by the negative results obtained with all the samples under validation trial. From the general farmers rearing, groups of larvae were collected from each farmer at the rate of 1 group of 15 larvae for each of 25 dfls. under rearing. Each group was subjected to diagnosis separately and the results were recorded group wise for each farmer. The intensity of infection was depicted by the number of group of samples/farmer positive to infection. The more the number groups per farmer is positive, the infection with farmers crop was considered high. The methodology adopted for the trials was similar to validation trial.

Prevention of spread of diseases in the rearing bed

To prevent spread of diseases in silkworm rearing, the silkworm body and their rearing seat were disinfected by application of silkworm body and rearing seat disinfectant - Vijetha. The disinfectant was dusted immediately after every moult before feeding and on the 4th day of final instar at the rate of 3 g/sq. ft (2nd and 3rd instars) and 5 g/ sq. ft (4th and final instars) bed area. During rainy and winter season, 2% of Dithane M45 in kaolin was also applied at the rate of 3 g/ sq. ft on the 3rd day of 4th instar and again on 2nd and 6th days of final instar.

In addition to the practice of silkworm disease control technology during the rearing, emphasis was also given to feed quality mulberry, providing congenial environment for silkworm and care in handling of mature silkworms to avoid injury to the larvae.

Results and Discussion

The results on validation trials of practical disease management conducted in different sericultural areas of Karnataka, Andhra Pradesh and Tamil Nadu are presented in Table 2. The result of practice of technology in rearing 845 farmers crops covering 149,530 bivoltine CSR hybrid layings during a period of 4 years reflected the effectiveness and potential of the technology. None of the farmer's

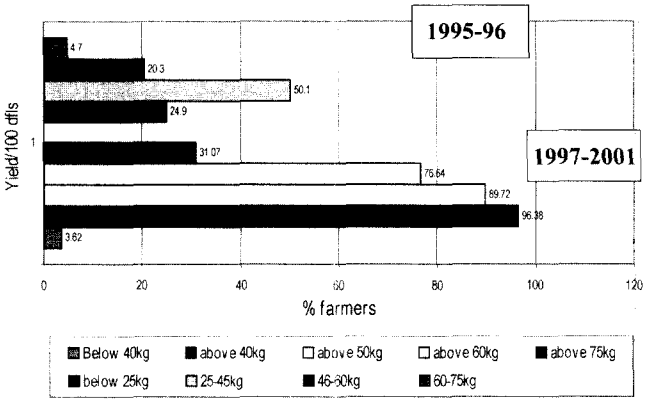

Fig. 1. Yield/100 dfls before and after demonstration of bivotine sericulture technology.

Table 2. Performance of disease control technology on cocoon crop performance in different sericultural areas

	Area	No. of farmers crop covered	Total no. of disease free layings	Mortality range due to diseases (%)		Cocoon yield/ 100 dfls. (kg)
				Pre cocoon stage	Post cocoon stage	
	Karnataka					
1	Srirangapatna	169	26,775	0.02 - 2.00	4.38 - 29.99	64.53
2	Halagur	133	17,000	0.01 - 4.00	0.64 - 26.00	65.33
3	Halasahally	54	7,775	0.01 - 1.00	0.73 - 8.00	74.83
4	Sira	146	33,025	0.80 - 4.00	2.00 - 28.50	66.64
5	Turuvekere	87	14,500	0.00 - 4.30	1.16 - 4.28	78.82
6	J.N.Kote	69	10,950	0.02 - 2.00	2.31 - 25.00	60.31
7	Kengeri	28	6,800	0.00 - 3.00	2.00 - 9.00	69.02
8	Varthur	33	8,900	0.10 - 0.20	1.54 - 8.66	76.25
	Andhra Pradesh					
9	Kuppam	54	11,100	0.00 - 3.77	0.50 - 8.00	80.62
	Tamil Nadu					
10	Gobichettypalyam	72	12,705	0.00 - 0.10	1.36 - 7.52	66.80
	Total/Range/Average	**845**	**149,530**	**0.00 - 4.30**	**0.50 - 29.99**	**68.98**

crop has failed due to disease and the average yield of cocoon was 68.98 kg/100 dfls. The technology ensured high percentage of larvae to survive and spin cocoon. 31.07% farmers crops yielded more than 75.00 kg cocoons/100 dfls (Fig. 1) and 76.64% farmers harvested above 60.00 kg cocoons/100 dfls. Only 3.62% of the farmers harvested less than 40.00 kg/100 dfls. which may be due to lapse in the practice of technology.

The performance of crops in terms of survival of larvae and yield with the same farmers prior to validation trial was significantly low. Hardly 4.70% of the farmers were getting > 60 kg/100 dfls. Only 20.30% farmers were getting between 45 and 60 kg/100dfls. During the demonstration period, 76.50% of farmers harvested > 60 kg/100 dfls. Only 3.62% farmers got less than 40 kg/100dfls.

The mortality due to diseases at the time of cocoon harvest ranged from 0.00 - 4.30%. It was due to nuclear polyhedrosis and flacherie. Nuclear polyhedrosis, which was reported to prevail to an extent of 33 - 53% (Nataraju *et al.*, 1998) at farmers level in Karnataka was at insignificant level and it was recorded just prior to spinning with only 3.79% farmers. Low incidence of flacherie was reported in isolated cases prior to spinning or on mountages. Sporadic low incidence of muscardine was recorded during winter season. The mortality during post cocoon stage ranged from 0.50 - 29.99%. The low incidence of diseases recorded during the pre cocoon stage has got enhanced at post cocoon stage, which may be attributed to improper handling of cocoons.

The examination of 3rd instar silkworms immediately after second moult by behavioral observation, microscopy and immunodiagnosis were negetive. The larvae were healthy as made out by behavioral observation. The microscopy and immunodiagnosis of undersized and unmoulted larvae also revealed absence of pathogen. However, in the general farmer's crop the examination of 3rd instar silkworms immediately after second moult, the immunodiagnosis gave different results with respect to different crops. Among 50 crops, 28 crops were positive to BmIFV infection. The observation on the performance of crops at later stage indicated that with the exception of 6 crops, all crops developed flacherie disease due to BmIFV. The infection level ranged from 3 - 32%. Among the 22 crops, which were negative to BmIFV infection in early instar, only 5 crops developed flacherie ranging from 3 - 5% in later stage. The remaining farmers harvested yield ranging from 60 - 70 kg among bivoltine farmers.

The successful harvest of cocoons in silkworm rearing may be attributed to efficacy of the disease management technology. The practice of technology through selection of silkworm rearing house for effective disinfection, use of suitable disinfectant, early diagnosis of diseases and use of bed disinfectant and hygiene maintenance helped in avoiding infection of silkworm and the spread of diseases. The system of chawki certification using microscopy and immunodiagnosis adopted in the technology has helped in initiating specific management practices. The negetive results obtained in the trials and good performance of such crops suggests the need of healthy chawki for good harvest. The primary reason for the crop failure or low productivity with the farmers was due to several reasons. Among them was the improper disinfection due to unsuitable, inferior quality and hazardous disinfectant and incomplete adoption of the disease prevention measures.

The separation of rearing house from dwelling place is most essential for disinfection with all disinfectants under use in sericulture to day. Formalin and bleaching powder are among them. These disinfectants were not able to provide successful harvest consistently. To address this problem, the technology has used a new disinfectant - Chlorine dioxide in combination with slaked lime (Balavenkatasubbaiah *et al.*, 1999). Chlorine dioxide which is available in stable form, effective at broader pH, suitable in all types of rearing houses is a user friendly disinfectant having no repulsive odor, non-hazardous and is least corrosive. Being non-hazardous, the disinfectant may be used in any type of rearing house as well as personal hygiene.

The prevention of spread of all diseases in silkworm rearing is another aspect of silkworm disease management. The non-hazardous characters of chlorine dioxide solution help in its efficient use in rearing and personal hygiene as against the hazardous formalin and bleaching powder solution. The bed disinfection to prevent the spread of diseases is another important aspect. There are several bed disinfectants in use in sericulture but all of them prevent the spread of one or two diseases. The new disinfectant Vijetha (Datta *et al.*, 1998) adopted in the technology prevents the spread of all diseases. The practical technology addresses all the factors leading to disease development in silkworm rearing at farmer level in India and the result confirms the suitability and adaptability of the technology.

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