

Evaluation of Different Bed Disinfectants against the Spread of Common Diseases in Silkworm, *Bombyx mori* L.

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(Received March 5 2003; Accepted May 22 2003)

Silkworm body and rearing seat disinfection is an integral part of effective silkworm rearing as it prevents the spread of various diseases through secondary contamination in the rearing bed. Many bed disinfectants are recommended by various research institutes in India. In the present study, eight bed disinfectants viz., Vijetha, Labex, Resham Jyothi, Sanjeevini, Suraksha, Reshamkeet Oushadh, Dithane M45 and Captan recommended by various Sericultural Research Institutions in the country against the spread of common diseases of silkworm were tested to determine their comparative efficacy. For the study, all the treatment batches were introduced with fixed number of specific diseased larvae so as to maintain a constant source of pathogen in the bed and then followed by treatment with respective bed disinfectants. Percent mortality/infection due to each disease was recorded to determine efficacy of bed disinfectant treatment against respective disease. The results show that all the bed disinfectants tested have considerable efficacy against the spread of various diseases in the rearing bed. However, Vijetha was distinctly superior in its efficacy against all four major diseases of silkworm. The study emphasize the role of bed disinfectants in silkworm rearing and the fact that they should be judiciously selected based on the efficacy of the bed disinfectant and the type of disease prevalent in a particular season and area.

Key words: Silkworm, *Bombyx mori*, Bed disinfectants, Grasserie, Flacherie, Muscardine, Pebrine

Introduction

Diseases in silkworms are the major constraint in achieving high silk productivity. Silkworm diseases are caused by microbial pathogens such as viruses, microsporidia, bacteria and fungi. In India, the annual crop loss due to silkworm diseases is to an extent of 30 – 40% (Vaidya, 1960; Janakiraman, 1961). The crop loss is mainly due to improper disinfection and unhygienic rearing conditions at the farmers level. During the course of silkworm rearing, there is every possibility of silkworm larvae to get infected either through contaminated mulberry leaf or through other sources of contamination (Baig *et al.*, 1990). When a few silkworms are infected, they spread the pathogen within the host population through different means such as vomit, excreta, hemolymph or directly from the skin leading to secondary infection. Unless some curative/preventive measures are adopted during the rearing, this may ultimately lead to the increase in disease incidence resulting in crop loss. But, so far no curative methods are found feasible to control the diseases in silkworms and hence the prophylactic measures are the only way to contain the diseases. As a routine prophylactic measure, the usage of bed disinfectants has been in regular practice in almost all the countries where sericulture is practiced.

Various authors have described the efficacy of different chemicals and fungicides against specific as well as all common silkworm diseases and eventually evolved a number of suitable bed disinfectant formulations for the control of common silkworm diseases through out the world. Aoki (1958) developed lime-cerisan mixture for the control of silkworm diseases. Hukuhara (1987) reported that in Japan, Pafusol and Japanese acid clay is used for the control of various diseases. Noamani *et al.* (1974) studied the efficacy of Papzol (mixture of paraformaldehyde and others) as bed disinfectant against viral diseases of the silkworm *Bombyx mori* L. Samson *et*

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al. (1986) reported the efficacy of Captan, Dithane M45 and formaline chaff against the spread of muscardine in silkworm population. Baig *et al.* (1989, 1993) reported the efficacy of a dust formulation consisting of paraformaldehyde, benzoic acid and lime against nuclear polyhedrosis and based on this study, a bed disinfectant, Reshamkeet Oushadh was formulated and after extensive field trials it was recommended for use in field against Grasserie and Muscardine. Subba Rao *et al.* (1992) reported that the dusting of lime-bleaching powder mixture (97 : 3) daily once as bed disinfectant is effective against grasserie and muscardine. Sohaf *et al.* (1994) studied seven fungicides for control of white muscardine disease of silkworm, *B. mori* and suggested that treatment with Captan and Foltaf at 3% concentration is effective against muscardine. Battacharya *et al.* (1995) recommended dusting of Labex, which is a mixture of commercial bleaching powder and slaked lime in the ratio of 3 : 97 for controlling silkworm diseases such as grasserie, and muscardine. Datta *et al.* (1998) reported that disinfection of silkworm body and rearing seat using an effective bed disinfectant such as Vijetha becomes essential to kill pathogens and prevent the spread of the diseases during silkworm rearing. Samson *et al.* (1998) recommended the use of Resham Jyothi, a wide spectrum bed disinfectant against different silkworm diseases over the silkworms in the rearing beds. Patil and Sharadamma (2000) recommended the use of a bed disinfectant "Sanjeevini" which is also known as powder "A" for the prevention and control of grasserie and flacherie. Patil *et al.* (2000) reported that a bed disinfectant "Suraksha" also known as powder "B" is effective against white muscardine of silkworm. Singh *et al.* (2000) reported that alternative day application of Reshamkeet Oushadh and formaline chaff was effective in prevention of nuclear polyhedrosis and muscardine diseases under normal conditions. Ran-

gaswamy *et al.* (2000) reported the results of screening of four fungicides, *viz.*, Dithane M45, Thiram, Cuman-L, and Kavach against *Beauveria bassiana* and suggested that Dithane M45 (2%) was effective against white muscardine. Sasidharan *et al.* (2002) reported the result of farmer level trial of bed disinfectant Resham Jyothi to prove its efficacy against incidence and spread of silkworm diseases.

Several bed disinfectants evolved by different sericultural research institutes in India are currently in use and many of them have become quite popular with sericulturists (Bhattacharya, 1991). Among the bed disinfectants, some are reported effective for all the common diseases, while others are effective against one or two specified diseases only. No information is available on the comparative efficacy of various bed disinfectants against the four major silkworm diseases. Hence, the present study has been undertaken at laboratory level to find out the comparative efficacy of the popular bed disinfectants which are readily available in the market *viz.*, Vijetha (Trade name "Vetcare Vijetha"), Reshamkeet Oushadh, Labex, Resham Jyothi, Sanjeevini, Suraksha, 2% Captan and 2% Dithane M45 against the four silkworm diseases.

Materials and Methods

Silkworm eggs of CSR2 × CSR5 hybrid, received from the Bivoltine Silkworm Breeding Laboratory of Central Sericultural Research and Training Institute, Mysore were reared to use as test material. V1 variety of mulberry leaf was utilized for the rearing and the standard rearing method was followed as recommended by Krishnaswamy (1978). Eight bed disinfectants were tested against the common diseases of silkworm *viz.*, Grasserie, Flacherie, Muscardine and Pebrine as indicated in Table 1.

Table 1. List of bed disinfectants recommended against the common diseases of silkworm in Indian Sericulture

Sl. no.	Bed disinfectants	Tested against diseases			
		Muscardine	Flacherie	Grasserie	Pebrine
1	Vijetha	+	+	+	+
2	Reshamkeet Oushadh	+	–	+	–
3	Labex	+	+	+	+
4	Resham Jyothi	+	+	+	+
5	Sanjeevini	–	+	+	–
6	Suraksha	+	–	–	–
7	2% Dithane M45	+	–	–	–
8	2% Captan	+	–	–	–

(+) = Recommended and tested against the disease.

(–) = Not Recommended against the disease hence not tested.

Introduction of infected larvae in healthy colony of silkworm batches

To ensure the availability of infected larvae for introduction in a healthy colony of silkworm, four groups, each of 300 newly ecdysed second instar larvae were kept and inoculated separately with *Nosema bombycis* (1×10^7 spores/ml), *Bombyx mori* nuclear polyhedrosis virus (1×10^7 POBs/ml), *Beauveria bassiana* (1×10^7 conidia/ml) and *Bombyx mori* infectious flacherie virus (10^{-7} dilution) + *Streptococcus* sp. bacteria (1×10^7 cells/ml) as causative agents of Pebrine, Grasserie, Muscardine and Flacherie diseases of silkworm respectively. For each pathogen, 3 ml of inoculum was used for 300 larvae. The inoculation was done *per os* except for *B. bassiana*, where the inoculation was done topically by spraying the inoculum over the larvae. The inoculum of specific pathogen used was from the stock maintained in the silkworm pathology laboratory of the Institute. Before the resumption of feeding after second moult, the diseased larvae with symptoms of specific disease were introduced in each batch of 100 larvae as mentioned below:

Pebrine, 4 in 96 healthy worms; Muscardine: 2 in 98 healthy worms; Grasserie: 6 in 94 healthy worms; Flacherie: 6 in 94 healthy worms.

The diseased larvae were introduced in all experimental batches to ensure the constant source of infectious agents in the colony and subsequently batches were subjected to treatment with different bed disinfectant. Another set of larvae, where the respective diseased larvae were introduced as mentioned above but without any bed disinfectant treatment were also kept to serve as inoculated control for comparison of results. The larvae of silkworm were reared under optimum conditions of temperature and humidity except in muscardine batches which were maintained at high humidity during all three rearing trials.

Schedule of dusting

Eight bed disinfectants were tested in the present study.

Out of them, six bed disinfectants *viz.*, Vijetha, Reshamkeet Oushadh, Labex, Resham Jyothi, Sanjeevini and Suraksha were readily available in the market and were procured and used for the experiment. The two fungicide formulations *viz.*, 2% Captan and 2% Dithane M45 were prepared in the laboratory after procuring Captan and Dithane M45 from the market. 2% concentrations were prepared by mixing 20 g of fungicide in 980 g of kaoline.

The bed disinfectants were dusted over the silkworm body and the rearing seat using a muslin cloth once after each moult, half an hour before the resumption of feeding and once on fourth day of fifth instar. During second and third instar, bed disinfectants were dusted at the rate of 3 g/sq ft. of bed area and during fourth and fifth instars the dusting was made at the rate of 5 g/sq ft.

Three replications were maintained for each treatment. During the rearing, mortality due to specific disease was recorded daily. In case of Pebrine, the cocoons formed by larvae were allowed for emergence of moth and the moths were individually homogenized and each homogenate was microscopically examined for the presence of spore stage of *Nosema bombycis*. The observations on the mortality due to different diseases were recorded based on the symptoms followed by microscopic examination. The percent disease and percent disease reduction over inoculated control was calculated for each treatment. Three rearing trials were conducted and the data was subjected to statistical analysis to find out C. D. at 5% level.

Results

The average data of three trials on the percent mortality due to grasserie in the batches treated with different bed disinfectants and percent disease reduction over inoculated control are presented in Table 2. Data indicate that all the bed disinfectants tested were effective in reducing the incidence of grasserie, however, the degree of efficacy

Table 2. Efficacy of different bed disinfectants against the spread of grasserie in silkworm rearing

Sl. no.	Treatment	No. of larvae/bed	% grasserie disease	Healthy larvae (%)	% disease reduction over control
1	Vijetha	100	10.00	90.00	84.67
2	Labex	100	21.10	78.90	67.53
3	Resham Jyothi	100	18.44	81.56	71.62
4	Reshamkeet Oushadh	100	16.10	83.90	75.22
5	Sanjeevini	100	20.55	79.45	68.37
6	Inoculated control	100	64.99	35.01	
	S. E. \pm		3.41		
	C. D. at 5 %		10.73		

Table 3. Efficacy of different bed disinfectants against the spread of white muscardine in silkworm rearing

Sl. no.	Treatment	No. of larvae/bed	% white muscardine disease	Healthy larvae (%)	% disease reduction over control
1	Vijetha	100	08.44	91.56	91.01
2	Labex	100	42.22	57.78	55.03
3	Resham Jyothi	100	21.44	78.56	77.16
4	Reshamkeet Oushadh	100	18.78	71.22	79.99
5	Suraksha	100	13.22	86.78	85.91
6	2 % Captan	100	11.00	89.00	88.28
7	2 % Dithane M45	100	09.00	91.00	90.41
8	Inoculated control	100	93.89	06.11	
	S. E. \pm		2.76		
	C. D. at 5 %		8.37		

varied with different treatments. Reduction in mortality due to grasserie was highest in batches treated with Vijetha (84.61%) followed by Reshamkeet Oushadh (75.22%), Resham Jyothi (71.62%) and Sanjeevini (68.37%). The efficacy of Labex was comparatively less with 67.53% reduction in the mortality due to grasserie against inoculated control where no treatment with bed disinfectant was applied after introduction of source of infection.

The data on the mortality due to muscardine in batches treated with different bed disinfectants presented in Table 3 clearly shows that all the bed disinfectant formulations were effective in preventing the spread of white muscardine in rearing bed. Vijetha was found to be most effective against white muscardine with 91.01% reduction in mortality closely followed by 2% Dithane M45 with 90.41% reduction in mortality. The percentage reduction in mortality in other treatments ranged between 88.28 and 55.03%. Among the treatments, lowest mortality reduction was noticed in the batches treated with Labex (55.03%).

The data on the efficacy of different bed disinfectant powders against flacherie presented in Table 4 shows that

all the bed disinfectant formulations were effective up to a certain extent in preventing the spread of flacherie caused by mixed infection of BmIFV and *Streptococcus* sp. bacteria in rearing bed. Vijetha was found to be most effective in controlling flacherie with 62.67% reduction in mortality. It was closely followed in its efficacy against flacherie by Labex with 57.08% mortality reduction followed by Sanjeevini with 51.36% mortality reduction and Resham Jyothi with 49.49% mortality reduction.

The data on the efficacy of different bed disinfectant formulations against the spread of Pebrine in the rearing bed are presented in Table 5 which shows that all the bed disinfectant formulations had limited efficacy in preventing the spread of pebrine in rearing bed. However, among the treatments, Vijetha was found to be comparatively more effective in reducing the spread of pebrine with 52.28% reduction in pebrine infection followed by Resham Jyothi (33.69%) and Labex (27.63%).

Discussion

Sanitation of silkworm rearing environment by disinfect-

Table 4. Efficacy of different bed disinfectants against the spread of flacherie in silkworm rearing

Sl. no.	Treatment	No. of larvae/bed	% flacherie disease	Healthy larvae (%)	% disease reduction over control
1	Vijetha	100	28.99	71.01	62.67
2	Labex	100	33.33	66.67	57.08
3	Resham Jyothi	100	39.22	61.78	49.49
4	Sanjeevini	100	37.77	62.23	51.36
6	Inoculated control	100	77.66	22.34	
	S. E. \pm		5.51		
	C. D. at 5 %		17.98		

Table 5. Efficacy of different bed disinfectants against the spread of pebrine in silkworm rearing

Sl. no.	Treatment	No. of larvae/bed	% Pebrine disease	Healthy larvae (%)	% disease reduction over control
1	Vijetha	100	09.55	90.45	52.28
2	Labex	100	16.00	84.00	27.63
3	Resham Jyothi	100	14.66	83.34	33.69
4	Inoculated control	100	22.11	77.89	
	S. E. \pm		1.32		
	C. D. at 5 %		4.58		

ing it with chemical disinfectants is essential for successful disease free rearing. Even after disinfection of the rearing house and its surroundings before the onset of rearing, there is every possibility for silkworm larvae to get infected during the course of rearing either through food or other sources of contamination. It is therefore essential to kill or inactivate these pathogens existing in rearing bed periodically, by applying chemical formulations, popularly known as bed disinfectants.

Among the four major diseases, which cause considerable damage to the sericulture industry, Grasserie caused by BmNPV is of great significance. Nataraju (1998) reported 43.63% prevalence of Nuclear polyhedrosis of the total disease incidence in field. The horizontal spread of the disease in a colony of silkworm larvae is through the hemolymph containing innumerable infectious polyhedral bodies which are liberated after rupturing the skin when the diseased silkworm larvae, by its natural tendency crawl aimlessly in the rearing bed. To inactivate BmNPV and to prevent the spread of grasserie in the rearing bed, a number of authors have recommended different bed disinfectants. Baig *et al.* (1993) recommended Reshamkeet Oushadh as an effective bed disinfectant against grasserie disease of silkworm. Labex is recommended against BmNPV by Bhattacharya *et al.* (1995). Datta *et al.* (1998) recommended a bed disinfectant formulation against all common silkworm diseases including Nuclear polyhedrosis. Resham Jyothi another broad spectrum bed disinfectant was also recommended by Samson *et al.* (1998). Patil and Sharadamma (1999) recommended Sanjeevini against grasserie disease in silkworm.

The present study indicated that Vijetha was most effective as bed disinfectant against grasserie disease followed by Reshamkeet Oushadh, Resham Jyothi, Sanjeevini and Labex. The percent reduction in the incidence of grasserie over control due to the application of above five bed disinfectants ranged from 84.67 to 67.53%, which is quite significant.

White muscardine is another important disease, which is most common during winter and rainy season in sericultural areas of south India. The disease is caused by a

parasitic fungus *Beauveria bassiana* (Bals.) Vuill. and spread to other silkworm by contact. The incidence of muscardine was reported to be 17.36 to 17.96% during 1993 (Anonymous, 1994). A number of bed disinfectants have been reported to be effective against white muscardine (Baig *et al.*, 1993; Bhattacharya *et al.*, 1995; Datta *et al.*, 1998; Samson *et al.*, 1998; Patil *et al.*, 2000). Present study clearly suggests that all the bed disinfectants tested against muscardine showed considerable efficacy in preventing the spread of the disease. However, Vijetha resulted in highest disease reduction (91.01%) over control followed by 2% Dithane M45 (90.41%). Results also suggest that Labex was comparatively less effective against muscardine with only 55.03% reduction in disease incidence over inoculated control. It is significant to note that the percent reduction in mortality due to muscardine over control was above 77% except in batches treated with Labex, which shows that application of these bed disinfectants effectively control the spread of muscardine disease in a silkworm population.

Flacherie is a syndrome caused by different types of pathogenic bacteria, non occluded viruses *viz.*, BmIFV, BmDNV or by the mixed infections of these bacteria and viruses. 14.97 to 39.73% incidence of flacherie was reported during 1990 – 93 (Anonymous, 1994) Four bed disinfectants *viz.*, Vijetha, Labex, Sanjeevini and Resham Jyothi as recommended by different authors against flacherie have shown considerable efficacy against the flacherie in rearing bed. Significant reduction in the incidence in flacherie (49.49 – 62.67%) was recorded in the batches treated with all the bed disinfectants, when compared to inoculated control. However, Vijetha was found to be most effective.

Pebrine, a serious disease of silkworm caused by microsporidian parasite is characterized by capability of its spore stage to transmit from mother moth to the offspring. The disease also spread through *per os* infection by ingesting contaminated mulberry leaf. The incidence of pebrine varied from 19.88 to 65.54% during 1990 – 93 (Anonymous, 1994), whereas it was only 2.32% as reported by Samson *et al.* (1990). Present study on the

comparative efficacy of Vijetha, Labex and Resham Jyothi suggest that Vijetha is most effective with 52.28% disease reduction when compared to other bed disinfectants where the reduction in disease ranged from 27.63 to 33.69%. It is also clear from the results that the efficacy of all the bed disinfectants tested is comparatively lower in respect of controlling pebrine disease when compared to control of other diseases.

It can be inferred from the study that the bed disinfectants play a major role in preventing the spread of silkworm diseases in silkworm rearing and consequently improving the productivity of cocoon crop. Vijetha, a bed disinfectant developed and recommended by Central Sericultural Research and Training Institute, Mysore is most suitable in preventing all silkworm diseases during silkworm rearing.

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