International Journal on Emerging Technologies 15(2): 56-61(2024)

ISSN No. (Print): 0975-8364
ISSN No. (Online): 2249-3255

Use of Bed Disinfectants in Sericulture Against Different Silkworm Pathogens of *Bombyx mori* L.

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(Received 26 June 2024, Revised 01 August 2024, Accepted 27 August 2024)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The silkworm, *Bombyx mori* being domesticated insect is susceptible to various diseases namely protozoan, viral, bacterial and fungal which causes heavy losses to the silkworm farmers by reduction of the cocoon yield. The silkworm diseases cannot be controlled but can be prevented to curb the secondary contamination in the rearing bed to other healthy silkworms. In order to prevent the silkworm diseases, the use of various bed disinfectants in recommended schedule is suggested to ensure the healthy growth of larva and bumper cocoon production. Many bed disinfectants *viz.*, Labex, Resham keetoushad (RKO), Sanjeevini, Suraksha, Resham jyothi, Vijetha etc. are currently being used to thwart different disease causing pathogens during silkworm rearing which is proving to be helpful for farmers to boost the silk production.

Keywords: Silkworm, Pathogen, grasserie disease, bed disinfectant, Labex, Vijetha.

INTRODUCTION

The diseases *viz.,* Pebrine, Grasserie, Flacherie, and Muscardine are known to cause the substantial damage to silkworms. Around 15-20 kg/100 dfls which is about 30% loss is caused by various diseases in silkworms (Selvakumar *et al.,* 2002; Chandrasekharan *et al.,* 2006; Balavenkatasubbaiah *et al.,* 2015). During silkworm rearing, *B. mori* becomes prone to various disease-causing organisms and silkworm larvae may get infected through contaminated leaves and other sources of contamination (Baig *et al.,* 1990, 1993; Doreswamy *et al.,* 2004). The proper disinfection and hygiene are the only ways by which we can kill these pathogens and prevent these diseases from spreading and multiplication to the healthy silkworms. The disinfection can be carried out by rearing room disinfectants (Formalin, chlorine dioxide, bleaching powder etc.) and bed disinfectants (Vijetha, RKO, Slaked lime etc.) widely used in the silkworm rearing environment. Apart from the chemical-based bed disinfects thrust is now given upon the botanical based disinfectants as they are eco-friendly, easily available with the farmers, high specificity against the pathogens and do not leave residual material in the rearing area during silkworm rearing. In order to enhance the silk production, need of the hour is to develop highly productive mulberry varieties (Islam *et al.,* 2022a, 2022b; Islam, 2023; Islam *et al.,* 2023a) and silkworm races which are immune to adverse climatic conditions and diseases (Jolly *et al.,* 1987). The current review article has made an attempt to explicate the information available about chemical and botanical based disinfectants for the benefit of large people in order to make sericulture sector more progressive to enhance the total cocoon yield.

Popular rearing bed disinfectants in use for silkworm rearing. Disinfection is the most important operation to be carried out before commencement of silkworm rearing (Qadir *et al.,* 2023) to keep rearing area germ free for smooth conducting of silkworm rearing. As manual dusting of bed disinfectants is unsafe, so power/battery operated duster has been developed by CSRTI Mysore which evenly spreads the disinfectants in less time over the body of silkworms (Dandin and Verma 2002). The NaOCl (0.01 %) and combination of 50% vim * 50 % lime and 40 % vim * 60 % lime has more potential for reduction of mortality in silkworms against diseases than other treatments (Shifa *et al.*, 2020). Shashidhar *et al.* (2018) (Table 1) recorded significantly low disease incidence of grasserie (0.10 - 0.08 %), muscardine (0.05 - 0.35%) and flacherie (0.25 - 0.15%) after application of Ankush vijetha green and slaked lime powder combination compared to bundh powder. Singh *et al.* (2023) studied the impact of bed disinfectants *viz.,* Labex, Vijetha, Sericillin and Amla powder on silkworm larva [NISTARI × (SK6×SK7)] and found Labex@ 5g/sq. ft. 30 minutes before feeding significantly improved all the cocoon parameters of silkworm. The significantly lowest larval and pupal duration was recorded in case of bed disinfectant Vijetha @ 5g/ sq. ft, also highest fecundity, hatching percentage and moth emergence was recorded when dusted with Vijetha @ 5g/ sq. ft

with respect to control (Surapwar *et al.,* 2019). The following bed disinfectants are currently in use for preventing the silkworm diseases during silkworm rearing.

Vijetha: It is an important body and rearing bed disinfectant effective against all silkworm pathogens. It is the first powder formulation for preventing all silkworm diseases and was developed by central Silk Board then licensed to Tetragon chemical Ltd., who started its production in 1996. It is cost effective and can be used in summer, rainy and winter seasons throughout the year having shelf life of one year. It is applied @ 3g/sq. ft. in first and 2nd instar and @ 5g/sq. ft in 3rd, 4th and 5th instar and its cost benefit ratio is 1:6 (Anonymous, 2022g, 2022f).

Reshom Keet Oushadh (RKO): The CSRTI, Mysore developed this first silkworm body and rearing bed disinfectant in the year 1986 and is effective against grasserie and muscardine disease in young and late age silkworms (Anonymous, 2022b). The cocoon yield increases by 7 kg/100 dfls by application of 3.25 kg RKO/100 dfls and its shelf life is six months from the date of manufacture. Its ingredients are slaked lime powder, benzoic powder, captan/diathane and formaldehyde (Subbarrao *et al.,* 1992). It must be applied 30 minutes before giving the feed after bed cleaning process. The cost benefit ratio is 1:6 (Anonymous, 2022c).

Slaked lime($Ca(OH)_2$): It is an effective colourless crystal/white powder disinfectant widely used in silkworm rearing bed and is cheap and effective against all the silkworm pathogens (Lakshmanan *et al.,* 2010) especially during rainy season with very high temperature.

Formalin chaff: It is an effective against viral, fungal and bacterial diseases occurring during rearing of silkworms (Shankar, 2003). In first and second instar larvae, third instar larvae, fourth instar larvae and fifth instar larvae concentration of 0.4%, 0.5%, 0.6% and 0.8% is applied during silkworm rearing respectively. It consists of 1 part of formalin and 10 parts of Paddy husk.

Sanjeevini: This disinfectant is developed by KSSRDI (1990) and is presently licensed to M/s Suraksha Bio chem. Pvt. Ltd, Bidar and is a season specific bed disinfectant for the control of silkworm diseases *viz.,* grasserie and flacherie during summer and rainy seasons. It is in powder form having shelf life of six months. After application of Sanjeevini the cocoon yield increases by 6 kg/100 dfls. It is dusted on larvae after every moult before feeding and after bed cleaning on 3rd and 5th day of fifth instar. About 4 kg (tray rearing) and 6 kg (shoot rearing) of sanjeevini is applied for 100 dfls. Its cost benefit ratio is 1:5 (Subbrarao *et al.,* 1992; Anonymous, 2022d).

Ankush: It was developed by CSRTI, Mysore in 2000 and is an eco-friendly botanical based disinfectant with non-hazardous chemicals in definite proportions. It is effective against pebrine, grasserie, muscardine, flacherie etc. The shelf life is one year and its dusting should be done after every moult and during 3rd and 5th day of final instar @3gm/sq.ft/100 dfls. The cost benefit ratio is 1:7 (Sharma *et al.,* 2008; Balavenkatasubbaiah *et al.,* 2014; Anonymous, 2022f).

Labex: It is highly effective bed disinfectant against grasserie and muscardine disease developed by CSRTI, Berhampore in 2005 and is made up of two locally available chemicals (97% slaked lime + 3% bleaching powder). About 4 kg labex/100 dfls is required during rearing. The all-India trail conducted by CSB placed labex at first rank. Its shelf life is six months from the date of manufacture. It is applied @ 3 g/sq. ft. after each moult 30 minutes before resumption of feeding and on the 4th day of fifth instar after bed cleaning. Its cost benefit ratio is 1:2.95 (Anonymous, 2022h).

Sericillin: It is developed by CSRTI, Berhampore in 2013 is a cost-effective body and bed disinfectant consisting of three chemicals (lime + bleaching powder + fungicide). It is mainly effective against muscardine and aspergillosis disease of silkworm. The dusting of sericillin is done on larvae after each moult 30-40 minutes before resuming the feed. The additional dusting on the 4th day of 5th instar may be done after bed cleaning. For 100 dfls about 3-3.5 kg of sericillin is needed during rearing. The cost benefit ratio is 1:6.4 (Chakrabarty *et al.,* 2013).

Resham Jyothi: It is an effective wide spectrum bed disinfectant developed by Silkwom Seed Technology Laboratory (SSTL), CSB, Kodathi, Bangalore against grasserie, bacterial flacherie, infectious flacherie, muscardine and pebrine. Dusting of Resham Jyothi is done after every moult before resuming feed and on the 4th day of fifth instar. The cost benefit ratio is 1:4.3 (Anonymous, 2022e).

Suraksha: It is a chemical-based season specific bed disinfectant effective against fungal disease of silkworm developed by KSSRDI in 1990 and is recommended during winter and rainy seasons highly effective against white muscardine. It is currently licensed to M/s Suraksha Bio chem. Pvt. Ltd, Bidar with a shelf life of six months. The method of application is simple, easy to adopt and appropriate to the farmers socio economic conditions. The application of suraksha enhanced cocoon yield by 8 kg/100 dfls. Before brushing it should be dusted on empty trays and after that on the newly hatched larvae followed by feeding after 30 minutes. It should be dusted after every moult before feeding and during 5th instar on 3rd and 5^{th}day after bed cleaning. The quantity of 4 kg (tray rearing) and 6 kg (shoot rearing) of suraksha is needed/100 dfls. The cost benefit ratio is 1:6 (Anonymous, 2022d).

Reshme Aishwarya: It is a chemical-based bed disinfectant developed during 2006 by the collaboration of KSSRDI and M/S Santhosh enterprises Pvt Ltd. Bangalore. It is economical, easy to use and appropriate for the farmers socio economic conditions and effective against all the diseases which significantly increased the ERR percentage. It is dusted on the newly hatched larvae followed by feeding after 30 minutes. Moreover, dusting is carried out after every moult before feeding and on 3rd and 5th day of 5th instar after bed cleaning. The quantity of 4 kg (tray rearing) and 6 kg (shoot rearing) of reshme aishwarya is needed/100 dfls. Its

dusting increases the cocoon production by 5 kg/100 dfls. The cost benefit ratio is 1:4 (Anonymous, 2022d).

Musgard: It is a season specific powdered bed disinfectant developed by KSSRDI in 2006 against the fungal diseases of silkworm in winter and rainy seasons having a shelf life of six months. Its dusting is done on newly hatched larvae followed by feeding after 30 minutes and after every moult prior to feeding and on the 3rd and 5th day of 5th instar after bed cleaning. For 100dfls the quantity of musgard required is 4 kg and 6 kg for tray and shoot rearing respectively. It increases cocoon yield by 8 kg/100dfls and has cost benefit ratio of 1:7 (Anonymous, 2022d).

SamrakshakIt is a chemical-based bed disinfectant developed by KSSRDI in 2011 and in 2012 licensed to M/s S.S. Associates, Bangalore. It is highly effective against grasserie, pebrine, flacherie, muscardine and aspergillosis. It should be applied on empty rearing trays before brushing and on the newly hatched larvae before feeding. Dusting should be carried out after each moult before feeding and on 3rd and 4th day of fifth instar after bed cleaning. For tray and shoot rearing, 4 kg and 6 kg of samrakshak/100 dfls is recommended respectively. It increases cocoon yield by 8 kg/100dfls after dusting and has cost benefit ratio of 1:6 (Anonymous, 2022d).

Table 1: Effect of bed disinfectants on silkworm diseases.

Treatments	Post Rainy (%)				Rabi (%)				Mean of two seasons (%)
	Grasserie	Flacherie	Muscardine	Total disease incidence	Grasserie	Flacherie	Muscardine	Total disease incidence	
T1	1.25(6.42)	2.20(8.53)	0.56(4.29)	4.01(11.55)	1.10(6.02)	1.70(7.49)	2.25(8.63)	5.05(12.99)	4.53(12.29)
T2	1.10(6.02)	2.17(8.47)	0.51(4.10)	3.78(11.21)	1.07(5.94)	1.65(7.38)	2.10(8.33)	4.82(12.68)	4.30(11.97)
T3	0.25(2.87)	0.05(1.28)	0.40(3.63)	0.08(1.62)	0.15(2.22)	0.35(3.39)	0.58(4.37)	0.49(4.01)	0.25(2.87)
T4	0.30(3.14)	0.06(1.40)	0.49(4.01)	0.10(1.81)	0.20(2.56)	0.40(3.63)	0.71(4.83)	0.60(4.44)	0.30(3.14)
S.Em.±	0.36	0.39	0.06	-	0.32	0.54	0.59	-	-
CD (0.01)	0.84	0.91	0.13	-	0.73	1.21	1.33	-	-
CV (%)	3.91	5.12	2.98	-	4.51	4.67	3.21	-	-

Botanical based extracts effective against different silkworm pathogens. The plant-based extracts are very useful as they do not leave any residual effect unlike chemical based disinfectants. The plant-based extracts which contains different components may inhibit the growth of pathogens or kill them to prevent the diseases (Nigam, 1982). Dhirwani *et al.* (2015) carried out study on various plant-based herbicides *viz.,* tulsi (*Ocimum tenuiflorum*), neem (*Azadirachta indica*), haldi (*Curcuma longa*), amla (*Phyllanthus emblica*) and ber (*Zizipus mauritiana*) on silkworm larva (CSR2 × CSR4) and recorded lowest mortality of 1% (amla) and 4% (tulsi) leaf powder respectively compared to control. Raj (1994) while evaluating the antiviral activity of different aqueous extract of *Psoralea corylfolia, Tribulus terrestris, Acacia suma* and *Caesalpinia coriaria* after fed to silkworm through mulberry leaves during third instar recorded highest activity in the *P. corylifolia* (800 ppm) by reducing the grasserie disease by 80 %. The mixture of turmeric + chalk powder in the ratio of 1:5 (1 kg/100 dfls) during silkworm rearing reduced the grasserie disease by 63.16 and 62.45 % (Manimegalai and Subramanian 1999) in summer and winter respectively. The different aqueous herbal extracts *viz., clipta prostrata, Cannamomum zeylenica, Punica granatum, Phyllanthus niruri and Acalypha indica* were analysed against flacherie and muscardine disease of silkworm. Among these herbal extracts the *C. zeylenica* and *A. indica* proved to be more effective against bacterial flacherie whereas the *P. niruri* and *E. prostrata* were found to be effective against muscardine disease (Rani *et al.,* 2016). Dileepkumar *et al.* (2018) after using the diethyl ether and ethyl acetate extract of seaweed, *Sargassum wightii* showed antifungal activity against silkworm pathogens. Among the tested extracts the maximum zone of inhibition (20mm) was recorded in diethyl ether treated batches @3mg/mL^{-1} against *Aspergillus flavus* and *Beauveria bassiana* (19.66 mm) (Table 2). Manimegalai and Chandramohan (2005) reported that under in vitro conditions the botanical, *Thuja orientalis* L. effectively inhibited the growth of *Bacillus thuringiensis* at concentration of 1000 ppm. Furthermore *T. orientalis* at concentration of 10,000 ppm reduced the mortality caused by 01-TAD-01 and 01-CHI-01 strains of *B. thuringiensis* by 30.03 and 36.00 % respectively compared to control. The antifungal activity of raw aloe vera gel (Fatima *et al.,* 2008) and Garlic, onion and ginger (Krishnaprasad *et al.,* 1979) against white muscardine, *B. bassiana* was studied and these botanicals significantly inhibited the fungal growth with respect to control. The anti-fungal activity of seeds of ajwain (*Carcum capticum*), roots of costus (*Saussurea lappa*) and lead wort (*Plumbago zeylanica*), rhizome of galangal root (*Alpinia officinarum*), stem of long leaf pine (*Pinus longfolia*), dried fruit of tamarind (*Tamarindus indica*) and stem of clicorice root (*Glycyrrhika glabra*) was also reported against *Aspergillus niger* (Ray and Majumdar 1974). The studies on the application of different aqueous extracts of medicinal plants *viz.*, *Adathoda vasica, Terminalia arjuna, Pongamia glabra, Phyllanthus niruri* and *Bougainvillea spectabilis* were carried out afterfed to silkworm with mulberry leaves against polyhedral bodies (Latha *et al.,* 2011) and among these *P. niruri* recorded significantly highest cocoon weight and shell weight than control. The similar type of work was done by Anitharani *et al.* (2022) who fortified mulberry leaves with *A. vasica* and *P. niruri* extract containing various secondary metabolites which have antimicrobial properties and fed it to silkworm, PM×CSR2 (Kolar gold). The highest cocoon weight,

shell weight, pupal weight and shell ratio was recorded in silkworm batch fed with methanolic extract (*P. niruri*) followed by others. Manjunath *et al.* (2020) after evaluating different medicinal plant extracts against flacherie disease of silkworm (PM×CSR2) found significant improvement in cocoon parameters fed with *P. niruri* extract fortified mulberry leaves. Furthermore, extracts of *Ocimum sanctum* (Kuntamalla *et al.,* 2015), *C. longa* (Chavan and Bhawane, 2016) and *Ziziphus jujuba* (Sunil and Chandrashekhar 2016) administered leaves after fed to silkworm larva recorded maximum cocoon parameters.

Table 2: Zone of Inhibition against fungal pathogens using various solvent extracts.

Seaweed extracts	*Aspergillus flavus*			*Nomuraea rileyi*			*Aspergillus Oryzae*			*Beauveria bassiana*		
	1mg/ml	2mg/ml	3mg/ml	1mg/ml	2mg/ml	3mg/ml	1mg/ml	2mg/ml	3mg/ml	1mg/ml	2mg/ml	3mg/ml
EA	-	11.66±0.6	18±1	-	13±1	14.33±0.6	-	12±1	13.66±0.5	-	14.66±0.5	19±1
DCM	-	12.66±0.5	14.66±0.5	-	10.66±0.5	12.66±0.5	-	10.33±1.5	12.33±0.6	-	13.33±1.1	16.66±0.5
DEE	9.66±0.5	16±1	20.33±0.6	7.66±0.5	12.66±1.1	15.33±0.5	-	11.66±0.5	14±1	11.33±1	17±1	19.66±0.5

CONCLUSIONS

The silkworm larvae like other living organisms also suffer from many threating diseases during rearing which effects their growth and incur losses to silkworm farmers in the form of poor cocoon production. The feeding of poor-quality mulberry leaves, fluctuation in temperature and relative humidity, poor ventilation in the rearing environment deteriorates the situation more which finally leads to the outbreak of various kinds of diseases likegrasserie, muscardine, flacherie etc. In order to prevent the secondary contamination caused by these diseases, the dusting of different bed disinfectants as per recommended schedule is ensured which maximizes the cocoon yield thereby enhancing the profitability of silkworm rearers. Although the chemical-based disinfectants have their shortcomings as they leave residual effects in the rearing area but still, they are very much effective to keep check on silkworm pathogens and enhance the healthy growth and development of larvae. In future more research on the development of efficient bed disinfectants, mostly herbal based should be carried out as they are eco friendly and will suffice our needs for carrying out sustainable silkworm rearing.

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How to cite this article: Tajamul Islam and Jasmeena Qadir (2024). Use of Bed Disinfectants in Sericulture Against Different Silkworm Pathogens of *Bombyx* mori L. *International Journal on Emerging Technologies, 15*(2): 56–61.