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Research article

DISINFECTION AND HYGIENE TECHNOLOGY USING ASTHRA AND ANKUSH FOR THE MANAGEMENT OF SILKWORM DISEASES

M. Balavenkatasubbaiah*, K. Chandrasekharan, S. D. Sharma, A. R. Narasimha Nayaka and B. B. Bindroo

Central Sericultural Research and Training Institute, Central Silk Board, Mysore – 570 008, Karnataka, India.
* Corresponding author e-mail: mbvsubbaiah@gmail.com

ABSTRACT: Disinfection and hygiene are integral part of silkworm, *Bombyx mori* L. rearing and plays an important role in the elimination of pathogenic microbes. Use of effective disinfectant for eliminating the pathogens from the rearing environment and use of bed disinfectant to disinfect the silkworm body and rearing seat are important aspects in prevention of diseases during rearing. Central Sericultural Research and Training Institute (CSR&TI), Mysore has developed two products *viz*., Asthra, a new spray disinfectant for disinfection of rearing house and appliances and Ankush, a user friendly bed disinfectant for prevention of spread of all common diseases. Asthra, is a chlorine based disinfectant and is effective against all common silkworm pathogens at 0.05% concentration. The disinfectant is found to be effective in disinfecting the contaminated rearing house and rearing appliances. This disinfectant was test verified at farmers' level and found effective. Similarly, Ankush which is effective in preventing the spread of silkworm diseases during rearing was also tested at the farmer's level and the results confirmed its efficacy. Disinfection and Hygiene technology was developed using Asthra and Ankush for the management of silkworm diseases and test verified and found effective. These two products were commercialized for large-scale production and showing wide acceptance among farmers.
Key words: Asthra, Ankush, Disinfection, Hygiene, Silkworm, *Bombyx mori* L., Technology.

INTRODUCTION

Centuries of domestication of mulberry silkworm, *Bombyx.mori* L. has resulted in loss of certain wild characters, which made it susceptible to infection by major pathogenic groups leading to diseases and crop loss. Among silkworm diseases, Grasserie, Flacherie, Pebrine and Muscardine are major diseases. It is established that the cause of disease is pathogen and the diseased silkworm extrudes pathogens into the rearing environment, which form the source for the spread of disease in the population. The diseases in silkworm are best prevented through effective disinfection and hygiene. Disinfection of rearing house and appliances before initiation of rearing with effective disinfectant and disinfection of silkworm body and rearing seat using effective bed disinfectant during rearing eliminates the persistent pathogens. Different disinfectants *viz*., formalin [1], bleaching powder [2,3], iodine compounds [4,5], Chlorine dioxide [6,7] have been recommended for disinfection of rearing house and rearing appliances. Similarly, different bed disinfectants *viz*., Labex [8], Reshamkeet Oushadh [9], Vijetha [10], Resham Jyothi [11] and Suraksha [12] have been suggested for prevention and spread of diseases during rearing. However, these disinfectants/bed disinfectants have certain limitations in their usage. To overcome these limitations, CSR&TI, Mysore has developed Asthra, a spray disinfectant for disinfection of rearing house, its surroundings and appliances [13] and Ankush, a user-friendly silkworm body and rearing seat disinfectant for prevention of spread of all common diseases during rearing [14]. In the present study, the efficacy of these products both at laboratory and field levels individually and the Disinfection and hygiene technology using these products was tested at in-house level and the results were discussed. These products are already commercialized for large-scale production and supply.

MATERIALS AND METHODS

Germicidal activity of Asthra against silkworm pathogens

Asthra, a Chlorine based disinfectant is jointly developed by CSR&TI, Mysore with M/s Seri-Gro Products, Bangalore. The germicidal activity of Asthra was tested *in vitro* against the silkworm pathogens *viz*., *Bacillus thuringiensis* (1×10^8 spores/ml) and *Beauveria bassiana* (1×10^8 conidia/ml).

1 ml of these pathogens was separately centrifuged and the pellets were suspended in different concentrations of Asthra solution (0.01, 0.02, 0.03, 0.04, 0.05, 0.10 and 0.50%) for different durations (10, 20 and 30 min) at room temperature. These pathogens were centrifuged and the pellet containing the pathogen were re-suspended in 1 ml of sterilized distilled water and tested for their viability in respective culture media and were observed for growth of bacteria/fungus. One inoculated control culture having bacteria/conidia with out exposure to treatment was maintained for comparative purpose.

The germicidal activity of Asthra was also tested *in vivo* against silkworm pathogens *viz*., BmNPV (1×10^7 POBs/ml), *Nosema bombycis* (1×10^7 spores/ml), *Bacillus thuringiensis* (1×10^8 spores/ml) and *Beauveria bassiana* (1×10^7 conidia/ml). These pathogens were exposed to different concentrations (0.03, 0.04, 0.05, 0.10 and 0.50%) of Asthra for different durations as mentioned above and used for bioassay to determine their infectivity. 1 ml of these pathogens except *Beauveria bassiana* were per orally inoculated to 100 silkworm larvae of third instar (immediately after II moult). Three replications were maintained for each treatment and reared for a period of 10 days. In case of *Beauveria bassiana*, the treated conidia were inoculated per cutaneously. Observations were recorded for disease development and mortality. Inoculated control for each pathogen without treatment was maintained for the comparison.

Efficacy of Asthra as disinfectant for disinfection of rearing house exposed with silkworm pathogens

Based on the observations of the results of *in vitro* and *in vivo* studies, 0.05% of Asthra solution was test verified for its effectiveness in disinfection of silkworm rearing house exposed with silkworm pathogens. The silkworm pathogens (BmNPV-1×10^7 POBs/ml, *Nosema bombycis* spores- 1×10^7 spores/ml, *Bacillus thuringiensis* spores- 1×10^8 spores/ml and *Beauveria bassiana* conidia -1×0^7 conidia/ml) were exposed in petriplates during disinfection of rearing house (disinfectant solution sprayed @ 1.5 litre/sq. m floor area of rearing house). The pathogens were re-collected at different intervals after disinfection (30min, 1, 3, 6, 12 and 24 h), centrifuged and collected in sterilized distilled water and were inoculated to the silkworms as described earlier. An Inoculated control for each pathogen without exposure to disinfection was also inoculated to silkworms for comparison of results.

Efficacy of Asthra as disinfectant for disinfection of Rearing applicances contaminated with silkworm pathogens

In case of tray disinfection, 5 ml of each pathogen mentioned above were sprayed individually on rearing trays separately and air-dried. These contaminated trays were disinfected by the Asthra solution (0.05%). In these disinfected trays, second instar silkworms (3 replications of 100 larvae each) were reared up to 15 days and observations on disease development and mortality during rearing was recorded. A control batch of silkworms reared on contaminated trays without disinfection was maintained for comparison.

Field-testing of Asthra as spray disinfectant for disinfection of rearing house and appliances

Field-testing of Asthra as a disinfectant at farmers level was conducted in different sericultural areas of Karnataka, Tamil Nadu and Andhra Pradesh. A total of 135 crops were tested involving 18,716 dfls. of rearing with Asthra disinfectant. The crops of 129 farmers involving 16,105 dfls. were taken as control using other disinfectants for comparison of results.

Efficacy of Ankush as bed disinfectant against the spread of diseases in silkworm rearing

In earlier studies, different user-friendly bed disinfectant formulations were tested for their efficacy against the spread of four common silkworm diseases *viz.*, Grasserie, Flacherie, Muscardine and Pebrine and identified Ankush as effective bed disinfectant [14]. In the present study, carrier larvae for Grasserie (6), Flacherie (6), Muscardine (2) and Pebrine (4) were introduced in a healthy population of 94, 94, 98 and 96 larvae respectively to ensure the constant source of infectious agent with dusting of Ankush as per the schedule. Another two sets of larvae were also maintained for comparison of results. In first set, where the respective diseased larvae were introduced but without dusting of bed disinfectant was kept as inoculated control. In second set, where the respective diseased larvae were introduced with Vijetha dusting, a ruling bed disinfectant. The rearing was continued up to spinning and recorded the diseases.

Field-testing of Ankush as bed disinfectant

Ankush as a bed disinfectant for the prevention of spread of diseases during rearing was tested at farmers level. A total of 50 farmers crops (6,796 dfls.) were covered with Ankush and 46 farmers crops (6,968 dfls.) were covered with Vijetha dusting as per schedule.

In house evaluation of Disinfection and hygiene technology using Ashtra and Ankush

In-house evaluation of the technology of Disinfection and hygiene using Asthra and Ankush was conducted for one year covering 6 silkworm crops of bivoltine double hybrid (735 dfls.). The following activities and schedule of disinfection was followed using Asthra for disinfection of silkworm house and appliances (Table 1).

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. • 1st disinfection of rearing house, its surroundings and rearing appliances with 0.05% Asthra solution.
5 days before brushing	• Cleaning and washing of rearing house. • Sun drying of appliances.
4 days before brushing	• Optional disinfection of rearing house, its surroundings and rearing appliances with 0.3% slaked lime solution.
3 days before brushing	• 2nd disinfection of rearing house, its surroundings and rearing appliances with 0.05% Asthra solution.
2 days before brushing	• Opening the windows of rearing house for aeration.
1 day before brushing	• Preparation of brushing.

During rearing, personal hygiene was maintained by Washing hands and feet with Asthra solution before entering the rearing house and also after attending the rearing. Asthra solution was also used for collection of diseased/dead worms in a basin and also for disinfection of contaminated vinyl sheet used for silkworm litter collection. Rearing hygiene was maintained by dusting Ankush on silkworm body and rearing seat as per the schedule given below (Table 2).

Table 2. Schedule of dusting of Ankush as bed disinfectant

S. No.	Stage of application
1	After I moult, half an hour before resumption of feed.
2	After II moult, half an hour before resumption of feed.
3	After III moult, half an hour before resumption of feed.
4	After VI moult, half an hour before resumption of feed.
5	3rd day during V instar.
6	5th day during V instar.

Ankush was dusted 3 - 4 g/ sq. ft. bed area and the quantity of Ankush used was around 6 kg for 100 dfls.

RESULTS

Efficacy of Asthra

In vitro results on the efficacy of different concentrations of Asthra against *B. thuingiensis* and *B. bassiana* is presented in Table 3 and results indicated the effectiveness of Asthra at concentrations of 0.03 % and above.

Efficacy of the different concentrations of Asthra against different pathogens of silkworm *viz.*, BmNPV polyhedra, spores of *Nosema bombycis*, and *Bacillus thuringiensis* and conidia of *Beauveria bassiana* are presented in Table 4. The results incidated that 0.03 % and above concentrations were effective against all the pathogens tested.

The results on the efficacy of Asthra (0.05%) as disinfectant for disinfection of rearing house and rearing appliances is presented in Table 5. 0.05% concentration of Asthra solution was found effective with minimum duration of 30 min exposure. The results on the field-testing of Asthra indicated an improvement of 11.25 kg/100 dfls. in cocoon yield by the use of Asthra over the other disinfectants used in sericulture. The disease incidence *viz*., Grasserie, Flacherie and muscardine were comparatively low in batches disinfected with Asthra (Fig. 1). The rearing results indicated that the Asthra disinfectant is suitable for disinfection and hygiene practice in sericulture.

Efficacy of Ankush

Laboratory studies on the efficacy of Ankush indicated its high efficacy in preventing the spread of diseases during silkworm rearing. The dusting of Ankush has resulted the reduction of diseases over inoculated control to an extent of 74.14, 80.56, 96.00 and 69.75% in Grasserie, Flacherie, Muscardine and Pebrine respectively.

The results clearly indicate that the disease reduction efficacy of Ankush is on par with Vijetha (Fig. 2). The data on the field trials of Ankush as bed disinfectant for the prevention of spread of diseases during rearing is presented in Fig. 3. The evaluation studies showed that an average of 60.75 kg cocoons/100 dfls. were harvested in Ankush dusted batches when compared to 60.07 kg yield/100 dfls. in Vijetha batches. The incidence of diseases was 3.072% in Ankush batches when compared to 3.304 % in Vijetha dusted batches.

In House evaluation of Disinfection and hygiene technology

The data on the In-house testing of technology is presented in Table 6. A total of 6 crops were covered with 735 dfls. The evaluation studies revealed that an average of 87.31 kg cocoons/100 dfls. Were harvested with superior cocoon characters and good market rate for harvested cocoons.

Table 3. *In vitro* testing on the germicidal activity of Asthra

Pathogen	Duration (min)	Concentration (%)							Inoculated Control
		0.01	0.02	0.03	0.04	0.05	0.10	0.50	
B. thuringiensis	10	+	+	-	-	-	-	-	+
	20	+	+	-	-	-	-	-	+
	30	+	+	-	-	-	-	-	+
B. bassiana	10	+	+	-	-	-	-	-	+
	20	+	+	-	-	-	-	-	+
	30	+	+	-	-	-	-	-	+

- : Effective ; + : Not effective

Table 4. *In vivo* testing on the efficacy of Asthra against silkworm pathogens

Pathogen	Duration (min)	Concentration (%)					Inoculated Control
		0.03	0.04	0.05	0.10	0.50	
BmNPV	10	-	-	-	-	-	+
	20	-	-	-	-	-	+
	30	-	-	-	-	-	+
N. bombycis	10	-	-	-	-	-	+
	20	-	-	-	-	-	+
	30	-	-	-	-	-	+
B. thuringiensis	10	-	-	-	-	-	+
	20	-	-	-	-	-	+
	30	-	-	-	-	-	+
B. bassiana	10	-	-	-	-	-	+
	20	-	-	-	-	-	+
	30	-	-	-	-	-	+

- :Effective ; + : Not effective

Table 5. Efficacy of 0.05% concentration of Asthra in disinfection of silkworm rearing house and rearing appliances

Treatment	Rearing house				Rearing trays			
	BmNPV	*N. b.*	*B. t.*	*B. b.*	BmNPV	*N. b.*	*B. t.*	*B. b.*
30 min	-	-	-	-	-	-	-	-
1 h	-	-	-	-	-	-	-	-
3 h	-	-	-	-	-	-	-	-
6 h	-	-	-	-	-	-	-	-
12 h	-	-	-	-	-	-	-	-
24 h	-	-	-	-	-	-	-	-
Inoculated control	+	+	+	+	+	+	+	+

-: Effective (No infection); +: Not effective (Positive infection)

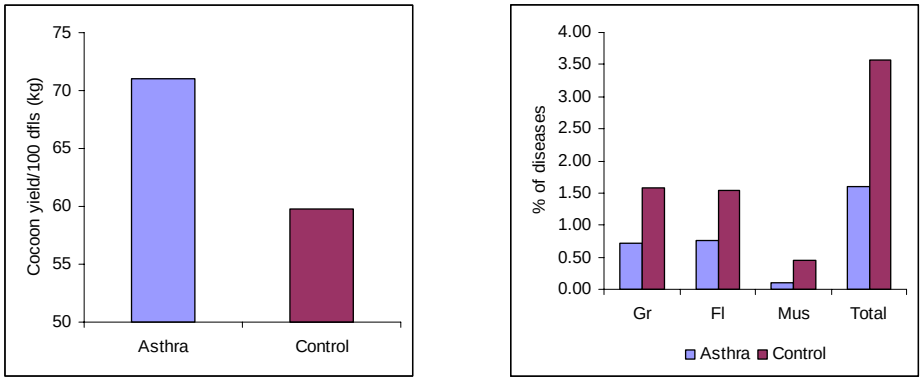

Figure 1: Performance of Asthra at farmers level

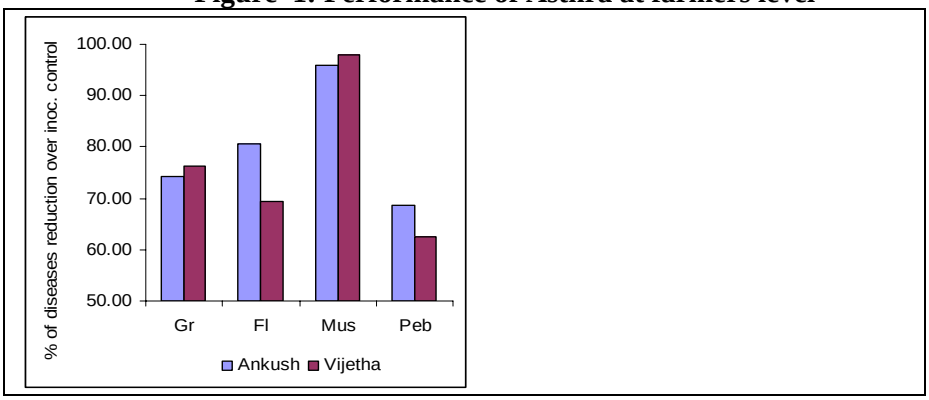

Figure 2: Efficacy of Ankush against the spread of diseases at laboratory level

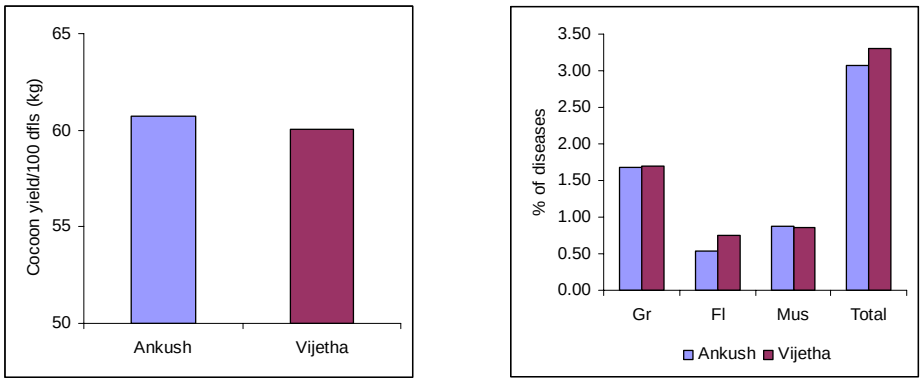

Figure 3: Performance of Ankush at farmers level

Table 6. In-house evaluation of Disinfection and hygiene technology using Asthra and Ankush

Crop No.	No. of dfls. reared	Single cocoon wt. (g)	Single shell wt. (g)	S. R. %	Yield/100 dfls. (kg)	Rate/kg cocoons (Rs.)
1	75	1.521	0.320	21.03	83.02	286.30
2	150	1.670	0.345	20.66	87.33	318.20
3	110	1.928	0.423	21.94	88.97	385.00
4	150	1.958	0.424	21.66	90.33	340.10
5	150	1.918	0.401	20.91	89.78	370.80
6	100	1.853	0.400	21.59	84.41	389.70
Total/Average	**735**	**1.808**	**0.386**	**21.29**	**87.31**	**348.35**

DISCUSSION

There are many disinfectants reported to be effective against silkworm pathogens. However, only a few of them are in practical use with several limitations associated with their use. The present study results clearly indicated that Asthra, a chlorine based disinfectant is effective against all the common silkworm pathogens. It is suitable and effective for disinfection of rearing house and appliances. The preparation of disinfectant is simple and easy. 50 g Asthra powder is required to prepare 100 liters of water (0.5 g/liter of water).

Advantages of Asthra over other known alternatives are:

- Asthra is effective in killing all silkworm pathogens in low concentration.
- The storage and transportation is easy as the disinfectant is available in powder form.
- It is less corrosive at the recommended concentration.
- Its solubility is high and hence, no sediment is formed therefore, no blockage in the nozzle of the sprayer.

Similarly, Ankush has the following advantages over the other bed disinfectants:

- Ankush prevents the spread of all common silkworm diseases
- It is a user-friendly substitute of existing bed disinfectants.
- It is an original indigenous mixture of user-friendly chemicals and plant product.
- Additional application of Anti-muscardine bed disinfectant is not required when using Ankush.
- Ankush is not having any pungent or unpleasant odour.

CONCLUSION AND RECOMMENDATION

The field trials conducted at farmers' level confirmed their efficacy of Asthra and Ankush. As a result, both these products were widely accepted by the farmers in a short period. To meet the demand of sericulturists, Ashtra was licensed to M/s Seri-Gro Products, Bangalore and Ankush to M/s Seri-Con Technologies, Bangalore (Ankush as trade name) for manufacturing and sale to the sericulturists. Due to subsequent heavy demand of Ankush, the license was given to four more reputed companies, *viz*., M/s Sericare, Bangalore (Trade Name : Ankush Vijetha Green), M/s Raibow Agro-vet Technologies, Kadapa, A.P (Trade Name : Rainbow Ankush Vijetha Gold), M/s Pure Chemicals Laboratories Pvt. Ltd., Bangalore (Trade Name: Ankush Green) and M/s Santhosh Enterprises, Bangalore (Trade Name : Santhosh Ankush Green) for large scale production and supply to sericulturists.

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