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Evaluation of a suitable silkworm bed disinfectant against silkworm diseases and survivability under temperate conditions of Kashmir

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Abstract
Crop loss due to the presence of silkworm diseases has been a regular feature of Indian sericulture. Diseases caused by various infectious pathogens viz., viruses, fungi, bacteria and microsporodia take a heavy toll of cocoon crops in the field every year. Improper disinfections, unhygienic rearing conditions result in wide spread contaminations due to various silkworm diseases. Therefore use of the bed disinfectants became a novel tool in the recent time to arrest this contamination in the rearing beds. In the present study the minimum disease incidence percentage of 51.80 percent was recorded in case of T_{10} (Vijetha), and the maximum disease incidence percentage of 85.76 percent was recorded by T_9 where no disinfectant was applied. The highest survival percentage of 45.00 percent was registered by T_{10} (Vijetha) followed by T_7 (Lime + Captan + Walnut hull) which recorded a survival percentage of 40.23 percent.

Keywords: pathogen, silkworm, disinfection, disease and survival

Introduction
Silkworm domesticated over centuries has become very delicate and susceptible to various diseases caused by microsporodia, bacteria, viruses and fungi (Doreswamy *et al*., 2004) [4] and in spite of strict adherence to rearing techniques, it is difficult to obtain desirable cocoon yield if diseases affect the silkworm. Diseases in silkworm are the major constraints in achieving high silk productivity. During rearing, silkworm larvae possibly get infected either through contaminated leaf or through other sources of contamination (Baig *et al*., 1990a) [1]. The infection by various pathogens and crop losses due to diseases is prevalent in all leading silk producing countries and is estimated to be about 15-20 kg per unit of 100 disease free layings which accounts for about 30 per cent of total loss (Selvakumar *et al*., 2002) [10]. Unless, some curative/ preventive measures are adopted during rearing, the infection built may lead to increase in the disease incidence and thus resulting in heavy crop loss. But so far no curative methods have been found feasible to control the diseases in silkworms and hence the prophylactic measures are the only way to control 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 being practiced. Adoption of proper and effective methods of disinfection and stepwise maintenance of hygiene helps to achieve the best results. Various bed disinfectants viz., Vijetha, lime, Captan, Dithane M-45, RKO (Resham Keet Oushad), Ankush, Vijetha green, Resham jyothi, Labex and Formalin chaff are used in silkworm rearing. Among these, the most commonly used bed disinfectants by the farmers are lime, hydrated lime and Vijetha which are being used in large quantities without any justification. Besides being costly, the chemical disinfectants have many limitations to be effective in open and rearing cum dwelling type of rearing house which results in incomplete disinfection and hygiene at farmer's level. Furthermore continuous use of these chemicals has been found to be carcinogenic to human beings as well. So under these circumstances formulation of new disinfectants having both the botanicals and effective chemicals in combination for the disease management becomes more essential.

Materials and Methods
The proposed investigation was carried out at Temperate Sericulture Research Institute

(TSRI), Mirgund during autumn 2014. Rearing of the hybrid (SH6 x JBEL) was carried out as per the standard rearing procedure (Dar & Singh, 1998) [5]. Different bed disinfectants were prepared as per the treatment details. Turmeric, which was used in the formulation of different treatments, was procured in the rhizome form. After washing it thoroughly with distilled water, it was shade dried for a week until it became dry and was then powdered and sieved and finally put in a sterilized conical flask and plugged with cotton to avoid external contamination. Similar procedure was also followed for walnut hull. The rest of the materials like Lime, Captan, Bavistin and Vijetha were procured in the packaged forms from the market. On the 2nd day of the 4th instar, the prepared formulations were dusted on the rearing seats/ trays and the worms were kept in the trays as such for one hour. Then the rearing trays containing the worms only (without the feed) were inoculated with the inoculum of Bombyx mori Nuclear Polyhedrosis Virus (BmNPV) @ 1x106 polyhedra/ml and the worms were left undisturbed for 24 hours. After 24 hours the formulations were again dusted over the trays and as such the dusting was done twice in the 4th instar and thrice in the 5th instar on the alternate days. Meanwhile the data pertaining to different parameters was recorded. Freshly prepared bed disinfectant formulations were used for all the trials. For every treatment four replications were maintained. Bed disinfectants were applied twice in the 4rth instar on alternate days and thrice in the 5th instar on alternate days. The powder was taken in a piece of thin muslin cloth and dusted uniformly on the larval bed and also on the empty space in the entire tray. No area in the tray was left without dusting. The dosage of different formulations used during the present investigation was 560g/1oo dfls after 3rd moult, 960g/100 dfls after 4th moult, 1540g/100 dfls after 3rd day of 4th instar and 1680g/100 dfls after 5th day of 5th instar.

Table 1: Details of different treatment combinations used in the experiment.

Treatment No.	Treatment detail
T1	Lime + powder
T2	Lime+Captan (99:1).
T3	Lime Powder+Bavistin(99:1)
T4	Lime+Turmeric Powder(99:1)
T5	Lime+Bavistin+Turmericpowder(98:1:1)
T6	Lime +Wood ash (98:2).
T7	Lime+Captan+Walnut hull powder (98.5:1:0.5)
T8	Lime+Turmeric powder+Cefixime (antibiotic) (98:1:1).
T9	Control 1 (without any application)
T10	Control 2 (Vijetha)

Disease incidence

Suspected silkworm samples were collected from each tray during each instar in perforated polythene bags and kept coded. The same samples were taken to the laboratory for microscopic examination to confirm the incidence of diseases, if any. The disease was calculated as per the formula adopted by Zargar, 2001.

$$\text{Disease Incidence (D.I)} = \frac{\text{No.of diseased worms collected}}{\text{Total no.of worms reared per replication}} \times 100$$

Larval survivability: The larval survivability was calculated by using the formula:

$$\text{Larval survivability (\%)} = \frac{\text{Total no.of survived larvae}}{\text{Total no. of larvae retained after the third moult}} \times 100$$

Experimental Findings

A. Disease Incidence percentage: The results of the experiment showed that the dusting of silkworm, Bombyx mori L. with T10 (Vijetha) recorded the lowest disease incidence percentage of 51.87 percent which was followed by the T7 (Lime+ Walnut hull+ Captan) which recorded the disease incidence percentage of 57.14 percent (Table 2). Statistical analysis of the data revealed that Vijetha was significantly superior to all other treatment combinations including T7.Among the prepared combinations T7 was statistically superior to all other treatments except T8 with which it was found to be at par (61.37%). The disease incidence percentage recorded by the other treatments include: T1-Lime (76. 28 %), T2–Lime + Captan (72.19%), T3-Lime+Bavistin (72.67%), T4– Lime + Turmeric (68.32%), T5 - Lime+ Turmeric+ Bavistin (65.18%) and T6 -Lime+ Wood-Ash-80 (29%) (Figure-1). The maximum disease incidence percentage was recorded in T9 – (85.76%) where no disinfectant was used. Also the application of T10 – Vijetha resulted in the highest percent reduction over control (39.4%) which was closely followed by T7 which recorded 33.03 percent reduction over control. The percent improvement over control recorded by other treatments include: T1 (Lime- 11.05%), T2 (Lime+ Captan-16.90%), T3 (Lime+ Bavistin-15.17%), T4 (Lime+ Turmeric-20.25%), T5 (Lime+ turmeric+ Bavistin-23.91%) and T6 (Lime+ Wood-Ash-6.27%) and T8 (Lime + Turmeric+ Cefixime-28.36%) respectively (Table-2).

Table 2: Effect of different disinfectants on the disease incidence percentage of mulberry silkworm.

Code	treatments	d.i %	reduction over control %
t_1	lime	76.28	11.05
t_2	Lime+Captan	72.19	16.90
t_3	lime+bavistin	72.67	15.17
t_4	lime+turmeric	68.32	20.25
t_5	lime+turmeric+bavistin	65.18	23.91
t_6	lime+woodash	80.29	6.27
t_7	Lime+Captan+Walnut hull	57.14	33.30
t_8	lime+cefixime+turmeric	61.37	28.36
t_9	no disinfectant	85.76	-
t_{10}	Vijetha	51.87	39.40
	cd ($p \leq 0.05$)	4.970	-

B. Survival percentage: The results of the experiment showed that the dusting of the T10 (Vijetha) recorded the highest larval survivability of 45.00 percent which was closely followed by T7 (Lime+ Captan + Walnut Hull) which recorded a larval survivability of 40.23 percent. Statistical analysis revealed that the T10 was superior to all other treatments, however, among the prepared combinations T7showed the best results. The lowest survivability of 11.27 percent was recorded by T9 where no disinfectants where used at all (Figure-4). The survivability percentage recorded by other treatments include: T1-Lime (20.05%), T2 -Lime + Captan (23.09%), T3-Lime + Bavistin (22.80%), T4 –Lime + Turmeric(27.58%), T5- Lime + Turmeric + Bavistin (31.05%), T6 -Lime + Wood-Ash (15.46%) and T8-Lime + Cefixime + Turmeric (33.23 %) respectively (Table –3).

Table 3: Effect of different disinfectants on survival percentage of mulberry silkworm.

code	treatments	Survival (%)
t1	lime	20.05
t2	Lime+Captan	23.09
t3	lime+bavistin	22.80
t4	lime+turmeric	27.58
t5	lime+turmeric+bavistin	31.05
t6	lime+woodash	15.46
t7	Lime+Captan+Walnut hull	40.23
t8	lime+cefixime+turmeric	33.23
t9	no disinfectant	11.27
t10	Vijetha	45.00
	cd ($p \leq 0.05$)	4.010

Discussion

Grasserie is one of the most common disease in silkworms caused by Bombyx mori nuclear polyhedrosis virus (BmNPV). The disease incidence ranges from 33-35% of the total diseases with higher incidence during summer and rainy seasons (Nataraju *et al*., 1998) [9]. The most commonly used method of preventing diseases in silkworm is the application of chemicals. However, due to the increased environmental awareness emphasis is now-a-days given on identifying non-chemical/ organic methods of disease management. In nature a number of plants have been found to harbor antiviral substances. In the present study various botanicals were used in conjugation with the already known chemical bed disinfectants such as Lime, Captan, Bavistin and Vijetha. Different combinations were made using these chemicals along with walnut hull, wood-ash and turmeric powder and accordingly seven combinations were formulated. T10 – (Vijetha) served as the positive control and T9- where no disinfectant was used served as the negative control. T7- (Lime+ Captan + Walnut hull) showed results almost similar to that of the positive control, even though T10 still proved to be better than the rest of the formulations. There was a significant difference in the incidence of nuclear polyhedrosis between the treatments. Apart from the relatively low incidence of polyhedrosis in the batches treated with the dust formulations of T7 (Lime+ Captan + Walnut hull) possibly reflects the better disinfecting action of walnut hull in combination with Lime and Captan (Table-2). Similar types of observations have been made by Baig *et al*., (1989) [2], Mannimegalai and Chandramohan (2005) [6], Clarke *et al*., (2006) [3], Sivaprakassam and Rabindra (1996) [11] and Manoharan (1996) [8]. This can also be attributed to the facts that allelochemicals and other phenolic compounds could be detrimental to nuclear polyhedrosis virus so that the silkworms can survive better. Though the highest survival percentage (45.00%) was recorded in case of T10 (Vijetha), among the prepared combinations T7 (Lime+ Captan + Walnut hull) provided the survival percentage of 40.23percent which was significantly superior to the rest of the prepared combinations. A significantly higher survival percentage in the batches treated with T7 (Lime + Captan + Walnut hull) suggests its greater efficacy over the other prepared combinations. In addition to this, this treatment did not leave any residual toxicity or phagodetterant effect thus allowing the worms to continue their growth and development without any adverse effect. The above findings are in line with those of Mannimegalai and Subramaniam (1999) [7] where the mortality rate was found to be least in the batches treated with plant based formulations. Sivaprakasam and Rabindra (1996) [11] in one of their studies has also made similar observations by reporting that allelochemicals from plants could be detrimental for nuclear polyhedrosis, so that the insects' ingesting the allelochemicals can survive better (Table-3).

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