Efficacy of Certain Antimicrobial Chemicals against Tasar Silkworm Disease Causing Pathogens

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ABSTRACT

Estimated crop loss due to Pebrine, Virosis, Bacteriosis and Muscardine is 20-25, 25-30, 10-15 and 2-5%, respectively in tasar culture. Different preventive methods by using chemical disinfectants, is the better option for the control of silkworm disease. Therefore, disease causing pathogens of tasar silkworm namely Cytoplasmic polyhedrosis virus (*AmCPV*), *Nosema mylittansis*, *Penicellium citrinum* and different types of bacteria were subjected for the *in vitro and In vivo* inactivation studies with four chemicals namely Sodium Silicate Pentahydrate (SSP), Tri-sodium orthophosphate (TSP), Benzalkonium chloride (BKC) and Didecyldimethylammonium chloride (DDC), which are known for the germicidal activity. *In vitro* results revealed that, 1.5-3% of TSP is capable in dissolution of *AmCPV* and suppression of bacterial and fugal pathogens at 2-5 minutes sterilization time. 1-3% of SSP has given good results in dissolution of *Nosema mylittansis* at 3-5 minutes. 1-5% of BKC and DDC have given best results in suppression of bacterial and fungal pathogens from 2-5 minutes sterilization time. *In vivo*, TSP treated lots have shown less mortality due to the various diseases except in pebrine disease when compared with other disinfectants treatments. In TSP treated batches, mortality percentage due to *AmCPV*, Pebrine spores, Bacteria and P. *Citrinum* recorded was 5%, 9%, 4% and 2%, respectively. SSP treated lots have shown low mortality due to Pebrine spore with 6%, where as mortality due to other diseases was higher than other disinfectant treatments. In the case of inoculated control mortality percentage was ranged from 53% to 98% where as in the case of health control no mortality due to the any diseases was found. In case of Virosis, percentile disease reduction, TSP occupied first place (94.90) followed by DDC (72.45), BKC (54.08) and SSP (46.94). SSP hold first rank in reduction of Pebrine disease (92.50). BKC and DDC have given moderate results in the suppression of all the diseases. The effective chemicals in the present study can be used for the formulation of broad spectrum disinfectant.

Key words: *Antheraea myllita* D., Disinfectants, Virosis, Pebrine

INTRODUCTION

In India, the extant of cocoon crop loss due to the silkworm diseases is nearly 40% (Sahay *et al.*, 2000). As there are no curative measures in silkworm rearing for the control of silkworm disease, different preventive methods using chemical disinfectants are practiced (Singh *et al.*, 2002). Thangavelu *et al*. (1995) used various chemicals as disinfectants for the control of different diseases in tasar silkworm. Bhattacharaya *et al.* (1995), Datta *et al.* (1998) used chemicals based body and rearing seat disinfectants like Labex, Sanjeevani, Vijetha, Resham Jyoti for management of diseases in mulberry silkworm Bombyx mori. Phenolic compound (Henga, 1977), Sodium hypochlorite and formalin (Vail *et al*, 1968) and formalin (Ignoff and Garcia, 1968) against several pathogens. Baig *et al.*, (1989) formulated a mixture of paraformaldehyde, benzoic acid and lime as a bed disinfectant against Nuclear polyhedrosis of mulberry silkworm. Bansal *et al.* (1996) tested Asiphore and sodium hypochlorite against virosis in tasar silkworm. T.K.O and Jeevan Suraksha are developed by CTR&TI is only confined to containment of bacteriosis and virosis diseases.

Information's are scanty on comparative efficacy of chemical disinfectants against different pathogens of tasar silkworm which can be utilized for the preparation of broad spectrum disinfectant. Hence, an attempt was made to test different chemicals against disease causing pathogens of tasar silkworm to find out efficacy. Based on in vitro studies, these chemicals can be utilized for making broad spectrum disinfectant.

MATERIAL AND METHODS

Based on the literature certain chemicals namely Sodium Silicate Pentahydrate (SSP), Tri-sodium orthophosphate (TSP), Benzalkonium chloride (BKC) and Didecyldimethylammoniumchloride (DDC) were selected for the study. Disease causing pathogens *AmCPV*, Microsporidia, Bacteria (*Bacillus, Micrococ* and *Serratia)* and *Penicellium citrinum* were taken for the study.

Preparation of chemicals concentrations: 1.0, 1.5, 2.0, 2.5, and 3.0% solutions of Sodium Silicate Pentahydrate, Tri-sodium orthophosphate, Benzalkonium chloride and Didecyldimethylammonium chloride were prepared by dissolving 1.0, 1.5, 2.0, 2.5, and 3.0 g of the same in 100 ml of sterile distilled water, respectively.

Isolation and purification of Pathogens: *N. mylittensis* were collected from pebrine infected silkworms and were purified by iso-density equilibrium centrifugation using percoll (Sato and Watanabe, 1980).

Cytoplasmic polyhedrosis virus (*AmCPV*): The polyhedra were purified following Aizawa, (1971) by repeated and differential centrifugation.

Procedure of *in vitro* study:

***AmCPV* and spores of *N. mylittensis*:**

One ml of the suspension of each pathogen of *AmCPV* and spores of *N. mylittensis* was centrifuged and the suspended pellet was exposed individually to 1.0 ml of the above mentioned concentrations of disinfectants for different durations viz. 1,2,3,4 and 5 minutes at room temperature (25 ± 1 °C). The suspended pathogen was centrifuged and the supernatant

was discarded. The traces of disinfectants were removed by washing the pellets twice in sterile distilled water by centrifugation. The final pellet was re-suspended in 1.0 ml of sterile distilled water individually and observed under phase contrast microscope for the structural alterations. The observations were recorded and analyzed.

Bactria and fungus

One ml of the suspension of each pathogen of bacteria and fungi having concentration of 1 $x10^{-7}$ cells/ml added to the1.0 ml of the above mentioned concentrations of disinfectants for different durations *viz.* 1,2,3,4, and 5 minutes at room temperature (25 ± 1 °C) and added in to sterile petri plates. There after melted and cooled (42-45°C) agar medium in case of bacteria and potato agar medium in the case of fungi was poured and mixed thoroughly by rotating the plates which were then allowed to solidify. Then the plates were incubated at 37°C for 24 to 48 hrs. and the observations were recorded and analyzed.

***In vivo* studies: Challenge with sterilized disease causative pathogens**

Disease causing pathogens were sterilized with selected chemicals separately for 5 minutes, tasar silkworms were challenged with sterilized disease causative pathogens and mortality was recorded. Inoculated and healthy controls were also maintained separately. In case of inoculated control the disease causing pathogens were feed to the silkworms without sterilization with the disinfectants, where as in the case of healthy control distilled water was sprayed on the leaf without pathogen load and silkworms were reared up to the cocoon formation.

RESULTS & DISCUSSION

Sodium Silicate Pentahydrate, Tri-sodium orthophosphate, Benzalkonium chloride and Didecyldimethylammonium chloride were tested for their efficacy against disease causing pathogens of tasar silkworm like, Cytoplasmic Polyhedrosis Virus (*AmCPV*) Microsporidia bacteria (*Bacillus, Micrococcus* and *Serratia*) and Fungi (*Penicellium citrinum*).

Growth of different Bacteria and Fungi in *in vitro* after sterilization (Table 1)

Sodium Silicate Pentahydrate: 1% to 2.5% concentration of Sodium Silicate Pentahydrate has not shown impact on growth of *Bacillus, Micrococcus, Serratia* and *Penicellium citrinum* at 1 to 5 minutes sterilization time. These bacteria and fungi have grown luxuriantly just like control. But, 4 and 5 minutes sterilization time of 3% concentration have shown partial growth of *Bacillus, Micrococcus, Serratia* bacteria and *Penicellium citrinum.*

Tri-sodium orthophosphate: 1% concentration of Tri-sodium orthophosphate has not shown great impact on growth of *Bacillus, Micrococcus, Serratia* and *Penicellium citrinum* at 1 to 3 minutes sterilization time. But, 1% concentration 4 and 5 minutes sterilization time have shown partial growth in case of *Bacillus, Serratia* bacteria and *Penicellium citrinum where as in the case of Micrococcus growth was observed in Micrococcus at* 4 minutes sterilization time. At the 1.5% concentration, partial growth of *Bacillus, Micrococcus, Serratia* bacteria and *Penicellium citrinum was observed* at 1 minute sterilization time whereas from 2-5 minutes sterilization time no growth was observed. From the concentration 2 to 3% no growth of bacteria and fungi was observed at any sterilization timings.

Benzalkonium chloride and Didecyldimethyl ammonium chloride: 1% to 3% concentration of Benzalkonium chloride and Didecyldimethyl ammonium chloride has shown no growth of *Bacillus, Micrococcus, Serratia* and *Penicellium citrinum* at 1 to 5 minutes sterilization time except 1% concentration 1 minute sterilization time which have shown partial growth.

Dissolution of *AmCPV* Polyhedra and Microsporidia (Table 2)

Sodium Silicate Pentahydrate: In all tried concentrations of Sodium Silicate Pentahydrate, no impact of chemical on dissolution of polyhedra of *AmCPV* at any sterilization timings was noticed. Sodium Silicate Pentahydrate could not able to bring any structural changes or alterations in the *AmCPV*. In the case of Microsporidea, dissolution was observed in 1to 3% concentration of the chemical in all 1 to 5 minutes sterilization timings, except in 1% concentration of the chemical in 1 and 2 minutes sterilization duration.

Tri-Sodium Orthophosphate: Complete dissolution of *AmCPV* was observed in 1.5 to 3% concentration of the Tri-Sodium Orthophosphate in all 1 to 5 minutes sterilization timings, except in 1.5% concentration of the chemical in 1 minute sterilization duration, where partial dissolution was observed. No dissolution of *AmCPV* was observed in 1.0% concentration of the Tri-Sodium Orthophosphate in all 1 to 5 minutes sterilization timings. In the case of Microsporidea, in all tried concentrations of Tri-Sodium Orthophosphate, no impact of chemical on dissolution of Microsporidea at any sterilization timings was noticed except in the 3% concentration of the Tri-Sodium Orthophosphate at 5 minute sterilization time where partial dissolution was observed.

Benzalkonium chloride and Didecyldimethyl ammonium chloride: 1% to 3% concentrations of Benzalkonium chloride and Didecyldimethyl ammonium chloride have shown no dissolution of *AmCPV* and Microsporidea was observed. Benzalkonium chloride and Didecyldimethyl ammonium chloride could not able to bring any structural changes or alterations neither *AmCPV* nor Microsporidea in all tested concentrations at all tested sterilization durations.

***In vivo* studies:**

Mortality of tasar silkworm (indoor) challenged with sterilized disease causative pathogens:

Results revealed that, TSP treated lots have shown less mortality due to the various diseases except in pebrine disease when compared with other disinfectants treatments. In TSP treated batches, mortality percentage due to *AmCPV*, Pebrine spores, Bacteria and P. *Citrinum* recorded was 5.00%, 9.00%, 4.00% and 2.00%, respectively. SSP treated lots have shown low mortality due to Pebrine spore with 6.00%, where as mortality due to other diseases was higher than other disinfectant treatments. BKC and DAC have shown low mortality due to the bacteria and *P.Citrinum* but mortality due to the *AmCPV* and pebrine spores was high. In the case of inoculated control mortality percentage was ranged from 53% to 98.00% where as in the case of health control no mortality due to the any diseases was found (Table 3).

Percentile disease reduction by the different disinfectants: In case of virosis percentile disease reduction, TSP stud first place (94.90) followed by DAC (72.45), BKC (54.08) and SSP (46.94). SSP hold first rank in reduction of pebrine disease (92.50). BKC and DAC have given moderate results in the suppression of all the diseases (Fig. 1)

Table1. Growth of Bacteria and Fungi in *in vitro condition,* after sterilization with different chemicals at different durations.

Disinfectant	**Conc. %**	**Sterilization time**																			
Pathogen		**Bacteria**															**Fungus**				
		Bacillus					***Micrococcus***					***Serratia***					**Penicellium citrinum**				
Exposer time (Minutes)		**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
Sodium Silicate Penta hydrate	1.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3.0	+	+	+	±	±	+	+	+	±	±	+	+	+	±	±	+	+	+	±	±
Tri-sodium orthophosphate	1.0	+	+	+	±	±	+	+	+	+	±	+	+	+	±	±	+	+	+	±	±
	1.5	±	-	-	-	-	±	-	-	-	-	±	-	-	-	-	±	-	-	-	-
	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Benzalkonium chloride	1.0	±	-	-	-	-	±	-	-	-	-	±	-	-	-	-	±	-	-	-	-
	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Didecyldimethyl ammonium chloride	1.0	±	-	-	-	-	±	-	-	-	-	±	-	-	-	-	±	-	-	-	-
	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+indicate positive, - indicate negative and ± indicate partial growth																					

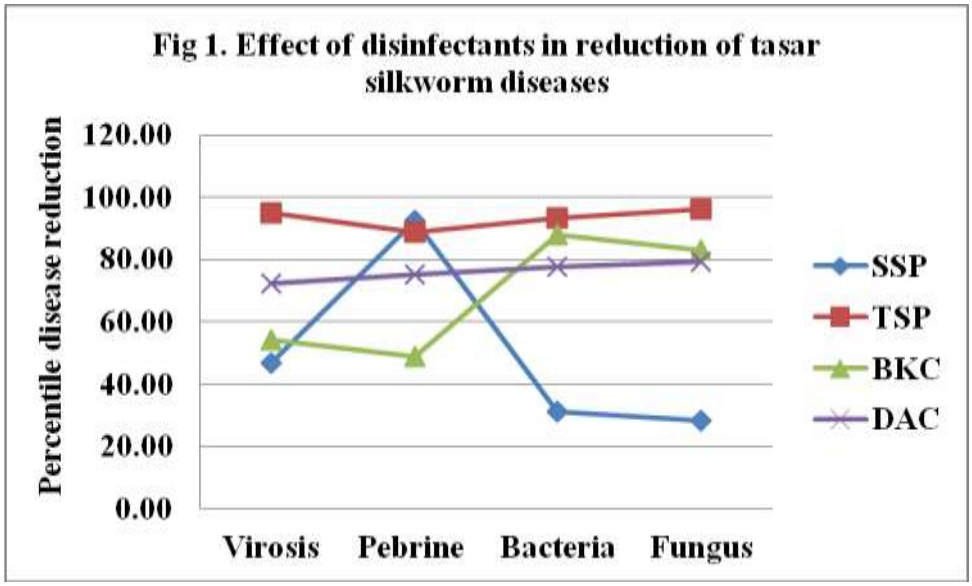

Table 2. Represents dissolution of polyhedra of *AmCPV* and Microsporidea.

Disinfectant	Conc. %	Dissolution of Pathogen									
		AmCPV Polyhedra					Microsporidia				
		Sterilization exposer time (Minutes)					Sterilization exposer time (Minutes)				
		1	2	3	4	5	1	2	3	4	5
Sodium Silicate Pentahydrate	1.0	-	-	-	-	-	-	-	+	+	+
	1.5	-	-	-	-	-	+	+	+	+	+
	2.0	-	-	-	-	-	+	+	+	+	+
	2.5	-	-	-	-	-	+	+	+	+	+
	3.0	-	-	-	-	-	+	+	+	+	+
Tri-sodium orthophosphate	1.0	-	-	-	-	-	-	-	-	-	-
	1.5	±	+	+	+	+	-	-	-	-	-
	2.0	+	+	+	+	+	-	-	-	-	-
	2.5	+	+	+	+	+	-	-	-	-	-
	3.0	+	+	+	+	+	-	-	-	-	±
Benzalkonium chloride	1.0	-	-	-	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-	-	-	-
	2.0	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
	3.0	-	-	-	-	-	-	-	-	-	-
Didecyldimethyl ammonium chloride	1.0	-	-	-	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-	-	-	-
	2.0	-	-	-	-	-	-	-	-	-	-
	2.5	-	-	-	-	-	-	-	-	-	-
	3.0	-	-	-	-	-	-	-	-	-	-

Table 3. Mortality of tasar silkworm (indoor) challenged with sterilized disease causative pathogens

Disinfectant	Mortality %			
	***AmCPV* ($1x10^4$ PIB/ml)**	**Pebrine spore (1×10^4 Sp/ml)**	**Bacteria ($1x10^{-4}$ cells/ml)**	***P. Citrinum* ($1x\ 10^4$ Sp/ml)**
Sodium Silicate Pentahydrate	52.00	6.00	40.00	38.00
Tri-sodium orthophosphate	5. 00	9.00	4.00	2.00
Benzalkonium chloride	45.00	41.00	7.00	9.00
Didecyldimethyl ammonium chloride	27.00	20.00	13.00	11.00
Control (I)	98.83	80.60	58.8	53.00
Control (H)	0.00	0.00	0.00	0.00

DISCUSSION

The results of the study indicated that, from the concentration 1.5% to 3%concentration of Tri-sodium orthophosphate has shown great impact on growth of *Bacillus, Micrococcus, Serratia* , and *Penicellium citrinum* (Fungus) at 1 to 5 minutes sterilization time. At the same time, complete dissolution of *AmCPV* was observed in 1.5 to 3% concentration of the Tri-Sodium Orthophosphate in all 1 to 5 minutes sterilization timings, except in 1.5% concentration of the chemical in 1 minute sterilization duration.

1% to 2.5% concentration of Sodium Silicate Pentahydrate has not shown impact on growth of *Bacillus, Micrococcus, Serratia* and *Penicellium citrinum* at 1 to 5 minutes sterilization time but in the case of Microsporidea, dissolution was observed in 1to 3% concentration of the chemical in all 1 to 5 minutes sterilization timings, except in 1% concentration of the chemical in 1 and 2 minutes sterilization duration.

1% to 3% concentration of Benzalkonium chloride and Didecyldimethyl ammonium chloride has shown no growth of *bacteria* and *fungi* at 1 to 5 minutes sterilization time that means these two chemicals are very good ion suppression of the *Bacillus, Micrococcus, Serratia* and *Penicellium citrinum.* 1% to 3% concentrations of Benzalkonium chloride and Didecyldimethyl ammonium chloride have shown no dissolution of *AmCPV* and Microsporidea was observed. Similar studies were conducted by the some of the researchers against various pathogens of silkworm and with the various chemicals. Anonymous, 1975 worked with the Formaldehyde and stated that Formaldehyde acts as a reducing agent by deoxidizing the pathogens and kills them, whereas bleaching powder releases nascent oxygen that has strong oxidizing action on the pathogens, chlorine changes the cell membrane to allow diffusion of cell contents and the alkaline calcium has strong germicidal action (Anonymous, 1975). Iwashita and Zhou (1988) reported that polyhedral bodies were dissolved quickly when dipped in saturated solution of calcium hydroxide and virions were inactivated. Similar types

of results have been observed in the present study. Balavenkatasubbaiah *et al.* (1994) observed that in slaked lime solution treatment, the polyhedral bodies of BmCPV of *Bombyx mori* were dissolved and inactivated.

In vivo studies, TSP treated lots have shown less mortality due to the various diseases except in pebrine disease when compared with other disinfectants treatments. SSP treated lots have shown low mortality due to Pebrine spore. BKC and DAC have shown low mortality due to the bacteria and *P.Citrinum* but mortality due to the *AmCPV* and pebrine spores was high. In case of virosis percentile disease reduction, TSP stud first place (94.90) followed by DAC (72.45), BKC (54.08) and SSP (46.94). SSP hold first rank in reduction of pebrine disease (92.50). BKC and DAC have given moderate results in the suppression of all the diseases. The effective chemicals in the present study can be used for the formulation of broad spectrum disinfectant for the containment of various diseases of tasar silkworm.

CONCLUSION

- At the concentration of 1.5%, Tri-Sodium Orthophosphate has given good results and could able to suppress the growth of the bacteria and dissolution of *AmCPV.*
- Sodium Silicate Pentahydrate is best for dissolution of microsporidia
- Benzalkonium chloride and Didecyldimethyl ammonium chloride chemicals are very good in suppression of the *Bacillus, Micrococcus, Serratia (Bacteria)* and *Penicellium citrinu (Fungal).*
- TSP stud first place followed by DAC, BKC and SSP In percentile disease reduction of virosis, Bactriosis and muscdine diseases. SSP hold first rank in reduction of pebrine disease.
- These chemicals or chemical combinations can be utilized for making disinfectant for the containment of various diseases of tasar silkworm.

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