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Efficacy of Disinfectants against Cytoplasmic Polyhedrosis Virus and Microspordia of Tasar Silkworm, *Antheraea mylitta* D.

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Bleaching powder solution (1 to 5%), slaked lime solution (0.1 to 0.5%) and formalin (1 and 2%) were tested for their efficacy against cytoplasmic polyhedrosis virus and *Nosema mylittansis* spores to control virosis and pebrine respectively in tasar silkworm, *Antheraea mylitta* in indoor rearing condition. All the disinfectants tested were found effective in suppressing the infection of virosis and pebrine significantly. Complete inactivation of *Antheraea mylitta* cytoplasmic polyhedrosis virus (AmCPV) was recorded when treated with 4% bleaching powder, 0.4% slaked lime for 20 min and 2.0% formalin for 30 min. Similarly treatments of 3.0% bleaching powder solution for 20 min and 2.0% formalin for 30 min were found effective in complete inactivation of *N. mylittanis* spores.

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

Introduction

Tasar silkworm, *Antheraea mylitta* D., an economically important insect is affected by several diseases. Virosis, pebrine, muscardine and bacteriosis are commonly prevalent diseases caused respectively by pathogens cytoplasmic polyhedral virus, *Nosema mylittansis*, *Penicillium citrinum* and different types of bacteria. Since curative measures are not found effective, different preventive methods are followed to protect the silkworms from diseases. Among different preventive methods followed, disinfections of cocoon preservation room, rearing field and appliances and maintenance of hygiene are the important and integral aspects in silkworm rearing. Among the diseases, virosis is very common which accounts for considerable loss to cocoon production. Sahay *et al.* (2000) reported incidence of virosis from 20 – 25% in tasar silkworm rearing. *N. mylittansis* is a well known entomopathogen of pebrine and loss incurred due to this disease varies from 35 – 40% (Sahay *et al.*, 2000). Several workers have tested different disinfectants and chemicals *viz.*, Phenolic compound (Henga, 1977), Sodium hypochlorit and formalin (Vail *et al.*, 1968) and formalin (Ignoff and Garcia, 1968) against several pathogens. Various disinfectants *viz.*, formaline (Kagawa, 1980), Asiphore (Venkata Reddy *et al.*, 1990), Chlorinated lime and hydrochloric acid (Miyajima, 1979), Chlorine dioxide (Balavenkatasubbaiah *et al.*, 1999) were tested against silkworm 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 hypochlorit against virosis and bacteriosis in tasar silkworm.

Information's are scanty on comparative efficacy of different disinfectants against virosis and pebrine of tasar silkworm. Hence, an attempt was made to test different disinfectants against cytoplasmic polyhedrosis virus (causing virosis) and microspordia (causing pebrine) to find out suitable disinfectants against these infectious diseases to fit well into the existing socioeconomic conditions of the tasar growers.

Materials and Methods

Disinfectants

The commonly used disinfectants *viz.*, Bleaching powder ($CaOCl_2$) containing 30% chlorine (DCM Sriram Consolidated Ltd., India), Slaked lime [Calcium hydroxide $Ca(OH)_2$] and formalin [aqueous solution of formalde-

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hyde (HCHO)], Hindustan Organic Chemicals Ltd., India.

1.0, 2.0, 3.0, 4.0 and 5.0% solutions of bleaching powder with 30% available chlorine were prepared by dissolving 1.0, 2.0, 3.0, 4.0 and 5.0 g of the same in 100 ml of sterile distilled water respectively. Slaked lime in 0.1, 0.2, 0.3, 0.4 and 0.5% was prepared by dissolving 0.1, 0.2, 0.3, 0.4, and 0.5 g of the same in 100 ml sterile distilled water respectively. 1 and 2.0% formalin was prepared from commercial formaldehyde (37% formaldehyde) by using the formula N1V1 = N2V2 where N1 is original concentration, V1 is original volume, N2 is required concentration and V2 is required volume.

Antheraea mylitta Cytoplasmic polyhedrosis virus (AmCPV)

Fresh cytoplasmic polyhedrosis virus inoculum was prepared from diseased silkworm. Completely whitened midgut obtained from cytoplasmic polyhedrosised silkworm at an advanced stage of infection were homogenized in sterile distilled water. The polyhedral suspension was filtered through a cheese cloth and the filtrate was centrifuged at 3000 rpm for 15 min and the polyhedra were purified following Aizawa (1971) by repeated and differential centrifugation. The resultant pellet suspended in distilled water was examined by light microscope for purity. The polyhedral suspension in sterile distilled water was prepared to contain 1×10^6 polyhedra/ml.

Nosema mylititansis spores

The spores of *N. mylittensis* were collected from pebrine infected silkworms and were purified by iso-density equilibrium centrifugation using percoll (Sato and Watanabe, 1980). The spore suspension containing 1×10^5 spores/ml was prepared in sterile distilled water.

Inoculation of pathogens and rearing of larvae

One ml of the suspension of each pathogen was centrifuged and the suspended pellet was exposed individually to 1.0 ml of the above mentioned concentrations of disinfectants for different durations *viz.*, 10, 20 and 30 min at room temperature ($25 \pm 1°C$). 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. The above pathogen's suspensions were smeared individually on to the Arjuna (*Terminalia arjuna*) leaves, air dried and fed to newly hatched tasar silkworm larvae of Daba eco-race. Untreated pathogen's suspensions smeared on to the arjuna leaves were fed to the larvae of control batches. Four replications with 50 silkworm larvae each were maintained separately for each pathogen and all concentrations of the disinfectants. Both treated and inoculated control batches were reared in indoor under normal rearing conditions up to spinning. The observations on development of diseases symptoms and larval mortality were made. The dead larvae in different treatments during rearing were examined microscopically for presence of concerned pathogens. Data recorded for mortality due to concerned pathogen were

Table 1. Percent of virosis infection after inoculation of POBs of AmCPV (1×10^6) treated with different disinfectants in tasar silkworm, *Antheraea mylitta* D.

Treatment	Conc. (%)	% of AmCPV infection duration of treatment (min)		
		10	20	30
Bleaching powder	1.0	42.00 ± 3.28	36.00 ± 2.18	22.00 ± 1.86
	2.0	35.00 ± 3.96	23.00 ± 1.32	18.00 ± 1.17
	3.0	20.00 ± 1.82	14.00 ± 1.15	6.00 ± 0.47
	4.0	4.80 ± 0.46	0.00 ± 0.00	0.00 ± 0.00
	5.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Slaked lime	0.1	58.00 ± 4.27	56.00 ± 3.82	53.00 ± 3.96
	0.2	39.00 ± 2.12	32.00 ± 2.96	26.00 ± 3.18
	0.3	19.00 ± 1.81	10.00 ± 0.92	4.00 ± 0.32
	0.4	3.75 ± 0.25	0.00 ± 0.00	0.00 ± 0.00
	0.5	000 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Formalin	1.0	28.00 ± 1.96	18.00 ± 1.08	12.00 ± 0.91
	2.0	2.00 ± 0.01	1.00 ± 0.01	0.00 ± 0.00
Inoculated control		91.50 ± 8.63	91.50 ± 8.63	91.50 ± 8.63
CD at 5 %		8.62	7.98	10.20

statistically analyzed using Completely Randomized Design (Snedecor and Cochran, 1971).

Results and Discussion

Effectiveness of disinfectants against *Antheraea mylitta* cytoplasmic polyhedrosis virus (AmCPV)

The results presented in Table 1 show that all the disinfectants tested were found effective in inactivating the AmCPV of tasar silkworm. The percent of inactivation of pathogen varied accordingly disinfectant used. Among all the disinfectants tested, slaked lime was found most effective even at low concentration. In 0.1% slaked lime treatment for 10 and 30 min 58.00 ± 4.27 and 53.00 ± 3.96% larvae respectively were infected with virosis (AmCPV), which were significantly lower ($P < 0.05$) than inoculated control (91.50 ± 8.63%). The infection of AmCPV decreased to 3.75 ± 0.25% at 0.4% concentration with treatment duration of 10 min. No infection of AmCPV was recorded in batches treated with 0.4% concentration with treatment duration of 20 – 30 min and 0.5% concentration with treatment duration of 10 – 30 min.

The microscopic examination of Polyhedral bodies revealed that the polyhedral bodies of AmCPV were dissolved when they were directly exposed to 0.5% slaked lime solution for 10 min and virions became inactivated as no mortality due to virosis in silkworms was recorded after feeding of this treated inoculum.

In 1.0% formalin treated batches, the infection of cytoplasmic polyhedrosis was recorded 28.00 ± 1.96% at 10 min and 12.00 ± 0.91% at 30 min treatment duration which was significantly lower ($P < 0.05$) than the inoculated control. In 2.0% formalin treated batches, infection of AmCPV was recorded 2.00 ± 0.01% at 10 min and 1.00 ± 0.01% at 20 min treatment duration whereas it was not noticed at 30 min treatment. 42.00 ± 3.28 – 22.00 ± 1.86%, 35.00 ± 3.96 – 18.00 ± 1.17% and 20.00 ± 1.82 – 6.00 ± 0.47% infections were recorded in 1.0, 2.0 and 3.0% bleaching powder treated batches respectively. In 4.0% bleaching powder treated batches 4.8 ± 0.46% infection was recorded at treatment duration of 10 min whereas no infection was recorded at 20 and 30 min treatment. Infection of AmCPV was not noticed in batches treated with 5.0% bleaching powder for 10 – 30 min.

Effectiveness of disinfectants against *Nosema mylitansis*

Results presented in Table 2 indicate that the different concentrations of bleaching powder and formalin were more effective in inactivating *N. mylittansis* spores, whereas slaked lime was not found very effective in inactivating the spores of *N. mylittansis*. In 1.0% bleaching powder treated batches, the pebrine infection was very low (10.50 ± 1.82% at 10 min and 5.0 ± 0.07% at 30 min treatment) and significantly different ($P < 0.05$) than the inoculated control (89.50 ± 12.57%). Infection of pebrine decreased to 2.56 ± 0.14% at higher concentration of 3.0% with treatment duration of 10 min. In 3.0% with 20 – 30 min treatment duration and 4.0% with treatment duration of 10 – 30 min, infection of pebrine was not

Table 2. Percent of pebrine infection after inoculation of pebrine spores (1×10^5) treated with different disinfectants in tasar silkworm, *Antheraea mylitta* D.

Treatment	Conc. (%)	% of pebrine in different treatment duration of treatment (min)		
		10	20	30
Bleaching powder	1.0	10.50 ± 1.82	6.30 ± 0.79	5.00 ± 0.07
	2.0	6.00 ± 0.69	4.36 ± 0.06	4.00 ± 0.05
	3.0	2.56 ± 0.14	0.00 ± 0.00	0.00 ± 0.00
	4.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	5.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Slaked lime	0.1	76.50 ± 9.26	74.20 ± 11.92	74.00 ± 9.56
	0.2	72.00 ± 7.59	76.62 ± 11.16	70.56 ± 7.18
	0.3	70.00 ± 8.02	72.01 ± 10.59	68.00 ± 5.24
	0.4	72.56 ± 9.16	68.00 ± 8.72	66.90 ± 5.89
	0.5	60.00 ± 5.23	58.00 ± 6.38	54.00 ± 3.98
Formalin	1.0	20.00 ± 2.06	16.00 ± 1.84	10.00 ± 0.92
	2.0	4.50 ± 0.82	2.00 ± 0.03	0.00 ± 0.00
Inoculated control		89.50 ± 12.57	89.50 ± 12.57	89.50 ± 12.57
CD at 5 %		9.67	11.52	10.96

noticed. 1.0% formalin was also effective and the infection of pebrine was 20.00 ± 2.06% in 10 min and 10.00 ± 0.92% in 30 min treated batches. In 2.0% formalin treated batches, infection of pebrine was 4.50 ± 0.82% at 10 min and 2.0 ± 0.03% at 20 min duration of treatment. The infection of pebrine was 0.00% at 2.0% formalin for 30 min duration of treatment. In slaked lime treated batches infection was comparatively higher than the bleaching powder and formalin treated batches, but it was significantly lower than inoculated control.

The results of the present study indicated that bleaching powder, slaked lime and formalin were significantly ($P < 0.05$) effective in the inactivation of both AmCPV and spores of *N. mylitansis*. 100% inactivation of AmCPV was observed only in 0.4% (20 – 30 min treatment duration) and 0.5% slaked lime, 2.0% formalin (30 min treatment duration) and 4.0% (20 – 30 min treatment duration) and 5.0% bleaching powder treated batches. Formalin acts as a reducing agent by deoxidizing the pathogens and kills them (JOCV, 1975), whereas bleaching powder in combination with water and weak acid (Co_2 in air) releases nascent oxygen that has strong oxidizing action on the pathogens, released chlorine changes the cell membrane to allow diffusion of cell contents outwards leading to the death of the pathogen, and the alkaline calcium which is liberated has strong germicidal action (JOCV, 1975).

Iwashita and Zhou (1988) reported that polyhedral bodies of nuclear polyhedrosis virus 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. The polyhedral bodies of AmCPV were dissolved and virions were inactivated when they were exposed to 0.5% slaked lime solution for 10 minutes. Balavenkatasubbaiah *et al.* (1994) observed that in slaked lime solution treatment, the polyhedral bodies of BmNPV of *Bombyx mori* were dissolved and inactivated.

Bleaching powder (3.0% or above) and formalin 2.0% were found very effective for inactivation of *Nosema* spores, whereas in case of AmCPV, slaked lime was found best for inactivation even at low concentration of 0.5%.

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