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Efficacy of Wood Ash as an Antiviral Agent against Cytoplasmic Polyhedrosis Virus of Tasar Silkworm, *Antheraea mylitta* D.

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The efficacy of wood ash from *Terminalia arjuna* (arjun) and *T. tomentosa* (asan) has been tested against virosis of tasar silkworm, *Antheraea. mylitta* D. The Polyhedral Occlusion Bodies (POBs) of Cytoplasmic Polyhedrosis Virus of *A. mylitta* (AmCPV) were exposed to the aqueous solution (0.5 to 4%) of wood ash for 5 to 30 minutes. The treated suspension of POBs was orally inoculated once to tasar silkworm larvae after 24 hours of 1st moult, and larvae reared in indoor on arjun leaves till spinning. The application of aqueous solution of wood ash has established its potential as antiviral agent against cytoplasmic polyhedrosis virus. Two percent aqueous solution of wood ash from *arjun and asan* dissolved the Polyhedral Occlusion Bodies (POBs) of cytoplasmic polyhedrosis virus of tasar silkworm and inactivated the virions within a short period of 20 to 30 minutes. *In vivo* efficacy of aqueous solution of wood ash resulted in reduction of larval mortality due to virosis. The mortality was reduced to 2.56±0.21and 3.03±0.32% when treatment of 2.0% solution of wood ash of arjun and asan respectively were applied for 20 minutes, compared to inoculated control (92.18±7.52%). No mortality was recorded when treatment of 2.5% solution of wood ash of arjun and asan were applied for 10 minutes or more.

Key words: *Antheraea mylitta*, silkworm, virosis, wood ash.

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Introduction

Tasar silkworms, *Antherea mylitta* reared on *Terminalia arjuna* (arjun) *T. tomentosa* (asan) and *Shorea robusta* is affected by several infectious diseases. Virosis, bacteriosis, pebrine and muscardine are the commonly prevalent diseases, which cause more than 40% loss to cocoon crop. Among the diseases, Virosis caused by cytoplasmic polyhedrosis virus is very common which accounts for 25-30% of the total cocoon crop loss (Sahay *et al.*, 2000) and thus pose a serious problem in sericulture. 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. Several workers have tested various disinfectants viz. formaline (Kagawa, 1980), Asiphore (Venkata Reddy *et al.*, 1990), Chlorinated lime and hydrochloric acid (Miyajima, 1979) and Chlorine dioxide (Balavenkatasubbaiah *et al.*, 1999) were tested against pathogens of mulberry silkworm, *Bombyx mori*. Baig *et al.* (1989) formulated a mixture of Para formaldehyde, benzoic acid and lime as a bed disinfectant against nuclear polyhedrosis of mulberry silkworm. Bansal *et al.* (1997) tested Asiphor and sodium hypochlorite against virosis and bacteriosis in tasar silkworm. Singh *et al.* (2005) tested some disinfectants against virosis and microspordia in tasar silkworm. These disinfectants are not economic and eco-friendly for use by the poor tribals of tasar culture and have many limitations to be effective in open and out door rearing, which results incomplete/ improper disinfections and hygiene at farmer's level.

Indian mythology is the important source of information on use of natural products to cure several ailments in human being and domestic animals. Wood ash is one among them. It is used frequently as a cleaning agent and disinfectant and is sprinkled over source of infection and

filthy materials in villages to kill germs in them. The wood ash suspended in water is highly alkaline and known for their antiviral action, however, have not been tested against the virus of tasar silkworm. Hence, an attempt was made to test the viricidal action of the wood ash against the cytoplasmic polyhedrosis virus of *Antheraea mylitta.*

Materials and Methods

The wood ash from *Terminalia arjuna* (arjun) and *T. tomentosa* (asan) was selected for the study as the twigs of arjua and asan end up as a fuel and ash is available in plenty with the tasar rearers. The 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% aqueous solution of wood ash was prepared by dissolving 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 g wood ash in 100 ml sterile distilled water.

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 cheesecloth and the filtrate was centrifuged at 3000 rpm for 15 minutes 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 1×10^5 polyhedra/ml.

Inoculation of POBs of AmCPV and rearing of larvae

One ml suspension of Polyhedra Occlusion Bodies (POBs) was centrifuged and the suspended pellet was exposed individually to 1.0 ml of different concentration (0.5 to 4.0%) of aqueous solution of wood ash for different durations viz. 5, 10, 15, 20, 25 and 30 minutes at room temperature (25 ± 1°C). The suspended polyhedra were centrifuged at 3000 rpm for 5 minutes and the supernatant was discarded. The traces of wood ash 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 suspension treated with 0.5 to 3.0% solution of wood ash was smeared individually on to the Arjuna (*Terminalia arjuna*) leaves, air dried and fed to newly hatched tasar silkworm larvae of Daba eco-race. The POBs of AmCPV treated only with sterile distilled water served as inoculated control. Four replications with 50 silkworm larvae each were

Table 1. Dissolution of polyhedral occlusion bodies of cytoplasmic polyhedrosis virus of tasar silkworm in wood ash solution

Treatment	Conc. (%)	Treatment duration (min.)					
		5	10	15	20	25	30
Arjun wood ash	0.5	+	+	+	+	±	±
	1.0	+	±	±	±	–	–
	1.5	+	±	–	–	–	–
	2.0	±	–	–	–	–	–
	2.5	±	–	–	–	–	–
	3.0	±	–	–	–	–	–
	3.5	±	–	–	–	–	–
	4.0	±	–	–	–	–	–
Asan wood ash	0.5	+	+	+	+	+	±
	1.0	+	+	±	±	±	±
	1.5	+	±	±	–	–	–
	2.0	±	±	–	–	–	–
	2.5	±	–	–	–	–	–
	3.0	±	–	–	–	–	–
	3.5	±	–	–	–	–	–
	4.0	±	–	–	–	–	–

+ No dissolution of POBs
± Partial dissolution of POBs
– Complete dissolution of POBs

maintained separately for all concentrations of aqueous solution of wood ash. Both treated and inoculated control batches were reared in indoor under normal rearing conditions up to spinning. The observations on development of disease symptoms and larval mortality were made. The dead larvae during rearing were examined microscopically for presence of POBs of AmCPV. Data recorded for mortality due to virosis pathogen were statistically analyzed using Completely Randomized Design (Snedecor and Cochran, 1971).

The polyhedral bodies exposed to wood ash solution were also examined under phase contrast microscope to observe their dissolution.

Results and Discussion

The results of action of aqueous solution of wood ash of arjun and asan on polyhedral occlusion bodies of cytoplasmic polyhedrosis virus are presented in Table 1 and Figs. 1, 2 and 3. The microscopic examination of treated Polyhedral bodies revealed that the partial dissolution of polyhedral occlusion bodies was started when treatment of 0.5% solution of arjun and asan was given for 25 and 30 minutes respectively. Complete dissolution of polyhedra was observed when they were directly exposed to 1.0% solution of arjun for 25 minutes and 1.5% solution of asan for 20 minutes.

The results presented in Table 2 show that aqueous solution of wood ash of arjun and asan were found effective in inactivating the cytoplasmic polyhedrosis virus of tasar silkworm. Inactivation percentage of cytoplasmic polyhedrosis virus increased with the increase in concentration of solution and duration of treatment. The mortality due to virus infection was 89.58±5.29 and 88.37±7.26% in treatment of 0.5% solution of wood ash of arjun and asan respectively for 10 minutes. The mortality was reduced to 2.56±0.21% in the treatment of 2% solution of arjuna for 20 minutes. Mortality due to virosis was not noticed in the treatments of 2.0% for 30 minutes and 2.5% or more for 20 and 30 minutes in case of arjun wood ash solution. Similarly, in case of asan, minimum mortality (3.03± 0.32%) in silkworm larvae was recorded in treatment of 2% for 30 minutes. Mortality was nil in treatment of 2.5% for 20 and 30 minutes. Mortality was recorded 92.18± 7.52% in inoculated control.

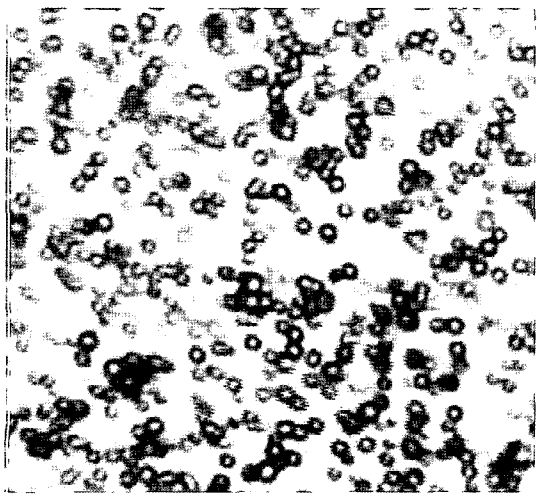

Fig. 1. Polyhedral bodies of AmCPV, Untreated.

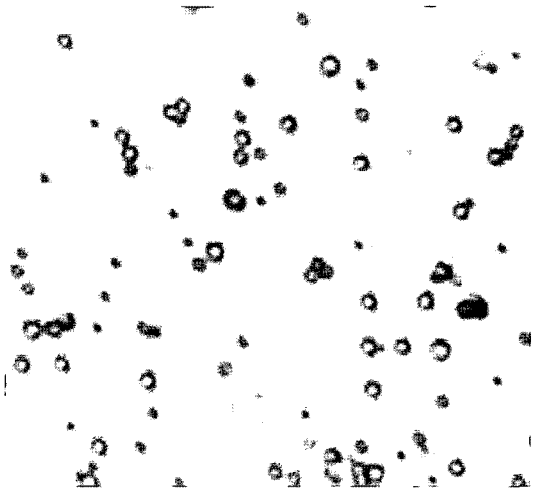

Fig. 2. Partially dissolved Polyhedral bodies of AmCPV after treatment with 1.5% aqueous solution of wood ash of Arjun for 15 minutes.

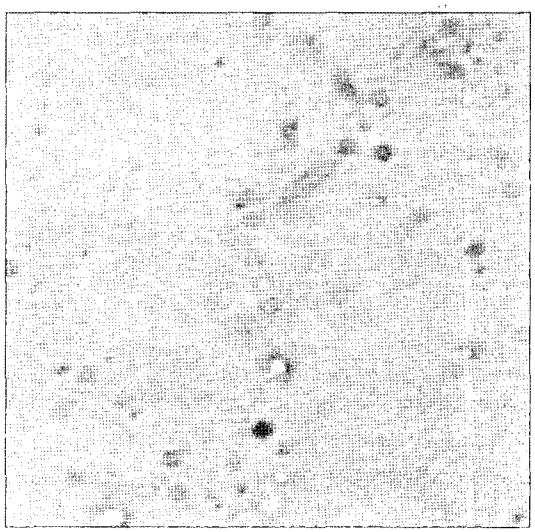

Fig. 3. Completely dissolved Polyhedral bodies of AmCPV after treatment with 2% aqueous solution of wood ash of Arjun for 20 minutes.

Table 2. Mortality in silkworm, *Antheraea mylitta* D. after inoculation with POBs (1×10^5) of AmCPV treated with aqueous solution of wood ash

Treatment	Conc. (%)	Mortality due to viroris (%)		
		Treatment duration (min.)		
		10	20	30
Arjun wood ash	0.5	89.58 ± 5.29	78.27 ± 4.38	71.14 ± 6.12
	1.0	61.08 ± 4.16	49.29 ± 3.22	23.85 ± 2.87
	1.5	42.12 ± 3.32	12.49 ± 2.16	2.83 ± 0.82
	2.0	9.25 ± 1.03	2.56 ± 0.21	0.00 ± 0.00
	2.5	8.62 ± 0.91	0.00 ± 0.00	0.00 ± 0.00
	3.0	9.00 ± 1.09	0.00 ± 0.00	0.00 ± 0.00
Asan wood ash	0.5	88.37 ± 7.26	82.18 ± 6.92	78.96 ± 5.11
	1.0	90.69 ± 8.09	77.53 ± 4.36	70.98 ± 3.97
	1.5	52.72 ± 3.88	28.59 ± 2.19	3.59 ± 0.35
	2.0	11.94 ± 1.16	3.61 ± 0.37	3.03 ± 0.32
	2.5	8.37 ± 0.92	0.00 ± 0.00	0.00 ± 0.00
	3.0	9.96 ± 1.15	0.00 ± 0.00	0.00 ± 0.00
Inoculated Control		92.18 ± 7.52	92.18 ± 7.52	92.18 ± 7.52
C.D. at 5%		8.42	7.56	5.68

The results of the present study indicated that aqueous solution of wood ash of arjun and asan were significantly ($P<0.05$) effective in the inactivation of cytoplasmic polyhedrosis virus of *Antheraea mylitta*. 100% inactivation of AmCPV was observed only in the treatments of 2% for 30 minutes and 2.5% or more for 20 and 30 minutes in case of arjun and 2.5% for 20 and 30 minutes in case of asan. These findings are more ore less similar to Kumar *et al.* (2000) who observed that the polyhedral bodies of nuclear polyhedrosis virus of mulberry silkworm dissolves and virions became inactive in aqueous solution of wood ash of mulberry and coconut. Thonchai and Sumanee (1987) have also tested wood ash against nuclear polyhedrosis virus and found that the polyhedral bodies dissolved and virions became inactive when they were directly exposed to the aqueous solution of wood ash. Similarly, Iwashita and Zhou (1988) Balavenkatasubbaiah *et al.* (1994) reported that polyhedral bodies of nuclear polyhedrosis virus of *Bombyx mori* were dissolved quickly when dipped in saturated solution of calcium hydroxide and virions were inactivated. Singh *et al.* (2005) have also observed that the polyhedral occlusion bodies of cytoplasmic polyhedrosis virus of tasar silkworm dissolved and became inactivated when exposed to the 1% solution of slaked lime. The efficacy of wood ash of arjun and asan as an antiviral agent against virus of tasar silkworm may be due to its high and stable alkalinity. Kumar *et al.* (2000) has reported the viricidal action of aqueous solution of wood ash of mulberry and coconut against the viruses of mulberry silkworm due to its high and stable alkalinity (pH 10.7-11.5).

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