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Screening of Medicinal Plants Against the Infection of A*ntheraea mylitta* Cytoplasdmic Polyhedrosis Virus (AmCPV) in Tropical Tasar Silkworm, A*ntheraea mylitta* Drury

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Ten numbers of plants, based on their medicinal value, were used to test their efficacy against virosis (caused by cytoplassmic polyhedrosis virus) in tasar silkworm, *Antheraea mylitta* Drury. in indoor rearing conditions. The aqueous extracts of leaf of *Azadirichita indica* (neem), *Acharanthus aspera*, *Psoralea corylifolia*, *Asparagus racemosus*, *Adhatoda zelanica* (Basak), *Andrographis paniculata* (Kalmegh), *Moringa oilephera* (sahjan), whole plant of *Phyllanthus urinaria* (Bhuiamla), *Centella asiatica* (Veng sag) and *Curcuma longa* (Haldi powder) in different concentrations were used for containment of virosis in silkworm larvae. The tasar silkworm larvae were orally inoculated with PIBs (1×10^6) of AmCPV in 2nd instar and treated with plant extracts in each instar (2rd instar onwards). The mortality due to virosis was recorded during larval period. The plant extracts, irrespective of their concentrations, were found effective in suppressing the virosis where *P. urinaria* reduced the virosis to 56.90% followed by *A. paniculata* (53.82%) and least in *C. asiatica* (5.15%). The lowest pooled larva mortality 36.99% was recorded in the treatment of *P. urinaria*. Comparatively higher larva mortality 39.91% was observed with the treatment of *A. paniculata*. The highest larva mortality in treatment was with *C. asiatica* (81.99%). In treated control larva mortality was 86.50%.

Key words: Medicinal plants, Cytoplasmic polyhedrosis virus, *Antheraea myllita.*

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Introduction

Tasar silkworm suffers with virus disease commonly known as virosis, caused by a cytoplasmic polyhedrosis virus (CPV) which causes 25~30% loss in cocoon crop (Sahay *et al.*, 2000). Chemical disinfectants and antibiotics have been used for managing the diseases in silkworm (Kagawa, 1980; Venkata Reddy *et al.*, 1990; Balavenkatasubbaiah *et al.*, 2000). In view of the high cost of the chemicals, antibiotics and their hazardous consequences, now a day's use of biodegradable materials like fresh plant extracts has been on the top priority for control of diseases in plants (Jesper and Ward, 1993) and animals (Satyavati, 1990). Use of chemical disinfectants for control of silkworm diseases is still a more risky management practice as it poses a problem of toxicity to silkworm. Use of plant products for control of grasserie (caused by nuclear polyhedrosis virus) in mulberry silkworm, *Bombyx mori* has been reported by Kumar *et al.* (1998) and Manimegalai *et al.* (2000). However, information's on use of botanicals against the infection of AmCPV in tasar silkworm are scanty. In the present study, an attempt has been made to test the botanical extracts for suppressing the virosis in tasar silkworm and increase the cocoon yield.

Materials and Methods

Ten numbers of medicinal plants known for their antibiotic properties were selected and tested for their efficacy against virosis in tasar silkworm, *A. mylitta*. The plants included leaf of *Azadirichita indica* (neem), *Acharanthus aspera*, *Psoralea corylifolia*, *Asparagus racemosus*, *Adhatoda zelanica* (Basak), *Moringa oilephera* (sahjan), whole plant of *Phyllanthus urinaria*, *Centella asiatica* and

Table 1. Mortality in silkworm, *Antheraea mylitta* D. after PIBs of AmCPV inoculation and treatment with plant extracts

Plants	Mortality due to virosis			% reduction from control		
	1st crop	2nd crop	Pooled	1st crop	2nd crop	Pooled
Azadirachita indica (P1)	76.16	71.49	73.82	15.67	19.56	17.16
Phyllanthus urinaria (P2)	38.33	36.16	36.99	57.56	56.25	56.90
Centella asiatica (P3)	84.66	79.33	81.99	6.26	4.03	5.15
Achyranthus aspera (P4)	81.33	77.33	80.66	9.96	6.45	8.20
Psoralea corylifolia (P5)	78.83	73.16	77.24	12.72	11.49	12.10
Asparagus racemosus (P6)	61.16	57.16	60.16	32.20	30.85	31.56
Adhatoda zelanica (P7)	43.99	41.16	42.58	51.29	50.20	50.74
Curcuma longa (P8)	42.83	39.66	41.24	52.58	52.02	52.29
Andrographis paniculata (P9)	41.16	38.66	39.91	54.42	53.22	53.82
Moringa oilephera (P10)	63.16	61.16	62.91	28.40	26.08	27.31
Conc. C1 (2%)	62.73	57.69	60.49	30.54	30.49	30.51
Conc. C2 (4%)	59.89	53.86	56.87	35.13	31.53	33.33
P1 × C1	78.00	70.33	74.16	13.64	14.92	14.28
P1 × C2	74.33	72.66	73.49	17.71	24.20	20.95
P2 × C1	40.66	37.00	38.33	54.98	55.24	55.11
P2 × C2	36.00	35.33	35.66	60.14	57.26	58.70
P3 × C1	84.00	78.00	81.00	7.00	5.64	6.32
P3 × C2	85.33	80.66	82.99	5.53	2.43	3.98
P4 × C1	82.66	76.66	79.66	8.49	7.26	7.87
P4 × C2	80.00	78.00	81.66	11.43	5.64	8.53
P5 × C1	80.00	72.00	78.50	11.43	12.90	12.16
P5 × C2	77.66	74.33	75.99	14.02	10.08	12.05
P6 × C1	63.33	56.66	61.99	29.89	31.46	30.67
P6 × C2	59.00	57.66	58.33	34.68	30.25	32.46
P7 × C1	45.33	42.33	43.83	49.81	48.79	49.30
P7 × C2	42.66	40.00	41.33	52.77	51.61	52.19
P8 × C1	44.66	40.00	42.33	50.55	51.61	51.08
P8 × C2	41.00	39.33	40.16	54.61	52.42	53.51
P9 × C1	42.33	40.00	41.17	53.13	51.61	52.37
P9 × C2	40.00	37.33	38.66	55.71	54.84	55.27
P10 × C1	66.33	61.66	63.99	26.56	25.54	26.05
P10 × C2	63.00	60.66	61.83	30.25	26.62	28.57
Inoculated control	90.33	82.67	86.50			
Plants CD 5%	10.39	9.86	9.92			
Conc. CD 5%	3.65	3.12	2.74			
Interaction CD 5%	6.26	5.37	6.42			

powder of rhizome of *Curcuma longa* (Haldi). Aqueous extracts of plant/ parts were prepared by grinding 50 g of clean and washed plant materials (leaf/bulb/rhizome) in a little water, soaked in 500 ml water for 10 hrs, filtered through double layered muslin cloth and cotton plugged neck of funnel. The filtered extract was concentrated to 50 ml using rotary evaporator (make of Buchi) at 40°C and 80 vacuum pressure. The plant extract so prepared was of 100% concentration (w/v) which was further diluted to desired concentration with distilled water before use.

***Antheraea myllita* cytoplasmic polyhedrosis virus (AmCPV) inoculum**

Fresh cytoplasmic polyhedrosis virus inoculum was pre-

pared from diseased silkworm, completely whitened midgut obtained from cytoplasmic polyhedrosised silkworms 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 using haemocytometer.

Inoculation of polyhedra inclusion bodies (PIBs) of AmCPV and treatment of larvae with plant extracts

200 ml suspension containing 1×10^6 polyhedra/ml was evenly smeared on to the arjuna (*Terminalia arjuna*) leaves, air dried and fed to 2nd instar larvae of Daba ecorace 24 hrs after moult. After 6 hrs of virus inoculation and once in 3rd, 4th and 5th instar, larvae were allowed to feed on arjuna leaves treated with 2.0 and 4.0% aqueous extract of plants. Three replications with 50 silkworm larvae each were maintained separately for each treatment and concentration. Both treated and inoculated control batches were reared in indoor under normal rearing conditions up to spinning. The plant extracts found effective in suppression of AmCPV infection in tasar silkworm more than 50% in indoor rearing have been tested in out door rearing condition for further confirmation of the results. The experiment was conducted during 1st crop rearing (July – August) and 2nd crop rearing (September – October). The observations were made on development of diseases symptoms, larval mortality and larval and cocoon parameters. The dead larvae in different treatments during rearing were examined microscopically for presence of polyhedra of AmCPV. Data recorded for mortality due to concerned pathogen, larval and cocoon parameters were statistically analyzed using Completely Randomized Design (Snedecor and Cockron, 1995).

Results

Effect of plant extracts on AmCPV infection and mortality in tropical tasar silkworm

Results of larval mortality and percent of reduction in virosis in silkworm, *A. mylitta* after inoculation with PIBs of AmCPV and treatment with plant extracts are presented in Table 1 and 2. The lowest larva mortality (36.00 ~ 40.66% and 35.33 ~ 37.00% during 1st and 2nd crop rearing respectively) was recorded in the treatment of aqueous extract of *P. urinaria* with different concentrations. Comparatively higher larva mortality (40.00 ~ 42.33% and 37.33 ~ 40.00%, 41.00 ~ 44.66% and 39.33 ~ 40.00%, 42.66 ~ 45.33% and 40.00 ~ 42.33%) was observed with the treatment of *A. paniculata, C. longa* and *A. zelanica* during 1st and 2nd crops respectively. The highest mortality within treatments was with *C. asiatica* (84.00 ~ 85.33 and 78.00 ~ 80.66 during 1st and 2nd crop respectively). In treated control larva mortality was 90.33 and 82.67% during 1st and 2nd crop respectively.

Table 2. Anova for larva mortality in silkworm, *Antheraea mylitta* D. after PIBs of AmCPV inoculation and treatment with plant extracts

Source of variation	Mean sum of square		
	Mortality Due to virosis (%)		
	1st crop	2nd crop	Pooled
Treatment	1711.422	1855.132	1783.27
Conc.	97.572	82.368	89.97
Treatment × Conc.	217.128	249.278	233.20
Treatment × Control	2681.18	2711.24	2696.21

Pooled analysis of data revealed 55.11 ~ 58.70% reduction in AmCPV infection in the treatment of *P. urinaria* followed by *A. paniculata* (52.37 ~ 55.27%), *C. longa* (51.08 ~ 53.37%) and *A. zelanica* (49.30 ~ 52.19%). The *C. asiatica* was least effective in reducing the AmCPV infection (3.98 ~ 6.32%). The plant extracts irrespective of their concentrations were found effective in suppressing the AmCPV infection where *P. urinaria* reduced 56.90% followed by *A. paniculata* (53.82%) and least in *C. asiatica* (5.15%).

The aqueous extract of 4 numbers of plants found effective in suppression of AmCPV infection more than 50% in indoor rearing, tested for confirmation of their efficacy in out door rearing and the results are presented in Table 3. All the plant extracts tested in out door rearing conditions have shown suppression of virus infection in tasar silkworm. Pooled mortality in larvae due to virus infection was recorded minimum (8.62 ~ 10.53%) with the treatment of aqueous extract of *P. urinaria.* The maximum mortality (14.47 ~ 16.17%) within the treatment was recorded with the treatment of *A. zeylanica.* The application of aqueous extract of *P. urinaria* (4%) was most effective in out door rearing which reduced the virus infection to the tune of 69.72%.

Effect of plant extracts on larva and cocoon parameters

The larval and cocoon parameters are presented in Table 4. The larva weight in fifth instar two days before spinning ranged from 35.27~40.61 g within different treatments. In control the larva weight was 34.12 g. Single cocoon weight, single shell weight and silk ratio percent of plant

Table 3. Larval Mortality and reduction in virosis after treatment with plant extracts(in outdoor rearing)

Plant extract	Conc. (W/V) %	Mortality (%)			Reduction of virus infection from control (%)		
		1st crop (July-Aug.)	2nd crop (Sept.-Oct.)	Pooled	1st crop (July-Aug.)	2nd crop (Sept.-Oct)	Pooled
P2	2.00	9.73*	12.33*	10.53*	62.98	59.76	63.01
	4.00	8.12*	10.11*	8.62*	69.11	67.00	69.72
P7	2.00	14.82*	17.51*	16.17*	43.63	42.85	43.20
	4.00	12.93*	16.00*	14.47*	50.82	47.78	49.17
P8	2.00	12.73*	15.11*	13.92*	51.58	50.68	51.11
	4.00	10.73*	13.33*	12.03*	59.19	56.49	57.74
P9	2.00	11.92*	14.56*	13.24*	54.66	52.48	53.57
	4.00	11.00*	12.38*	11.69*	58.16	59.59	58.86
Control		26.29	30.64	28.47			
Plants CD 5%		5.821	6.630	7.927			
Conc. CD 5%		2.960	2.347	2.267			
Interaction CD 5%		6.618	5.248	5.070			

*Significant at 5% than control

Table 4. Effect of plant extracts on Larval and cocoon parameters in silkworm in outdoor rearing

Treatment	Conc. %	Larval weight (g)	Cocoons harvested (%)	Cocoon wt. (g)	Shell wt. (g)	SR %
P2	2.00	39.51*	47.27*	13.98	1.59	10.87
	4.00	40.61*	50.00*	13.57	1.54	11.25
P7	2.00	36.00	40.84	12.30	1.37	10.33
	4.00	36.80	41.54	12.04	1.43	10.90
P8	2.00	35.27	39.99	12.05	1.41	10.96
	4.00	36.95	40.96	11.89	1.44	11.25
P9	2.00	40.12*	44.96*	13.77	1.52	10.37
	4.00	39.64*	46.03*	13.58	1.49	10.49
Control		34.12	38.37	12.49	1.41	11.00
CD 5%		2.95	4.91	1.69 (N.S.)	1.35 (N.S.)	1.05 (N.S.)

*Significant at 5% than control
N.S. - Non significant

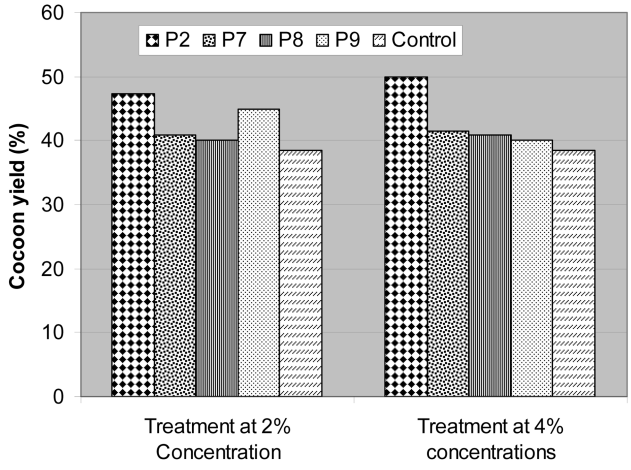

Fig. 1. Effect of plant extract on cocoon yield (%) in tasar silkworm, *A. myllita.*

extract treated batches were on par with the control which indicated that the plant extracts have no adverse effect on the silkworm larvae. Cocoon yield was highest 50% with the treatment of *P. urinaria* at 4% concentration followed by 46.03% with the application of *A. paniculata* at 4% concentration. The lowest cocoon harvest 38.37% was recorded in control (Fig. 1).

Discussion

In the present study all the plant extracts tested, except *C. asiatica, A. aspera* and *P. corylifolia*, were found effective in reducing the AmCPV infection significantly ($P < 0.05$) than treated control (Table 2). Plant extracts have also

shown significant difference ($P < 0.05$) with each other in reduction of virus infection in tasar silkworm. The treatments of plant extract of *P. urinaria, A. paniculata, C. longa* and *A. zyelanica* reduced the virus infection 50.74 ~ 56.98% in silkworm in indoor and 49.17 ~ 69.72% in out door rearing. Kumar *et al.* (1998) recorded a considerable reduction in grasserie diseases (caused by a nuclear polyhedrosis virus) in mulberry silkworm, *Bombyx mori* with oral feeding of crude aqueous extract of Curcuma longa (turmeric powder) and *Phyllanthus niruri*. Similarly Manigagalai *et al.* (2000) observed 63% reduction in grasserie disease in *B. mori* with the application of turmeric and chalk powder. The plant extracts tested in the present investigation have not been reported earlier against the virus disease in tasar silkworm.

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