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[Research]

Susceptibility of three Eco-races of tropical Tasar silkworm to *Antheraea mylitta* cytoplasmic polyhedrosis virus (AmCPV)

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ABSTRACT
Pathogenic infection in tasar silkworm, *Antheraea mylitta* Drury is common and there is a breed specific response regarding tolerance. Observations reveal the possibility of differential response by haemocytes to microbial infection in different breeds. Hence, the susceptibility of three eco-races of tasar silkworm *viz.* Daba, Sarihan and Raily to *Antheraea mylitta* Cytoplasmic Polyhedrosis Virus (AmCPV) infection and difference in total haemocyte counts were tested. The survival of Daba, Sarihan and Raily eco-races was significantly different ($p < 0.05$) when challenged with the same concentration (1×10^5 polyhedra/ml) of AmCPV. Daba eco-race was more tolerant to the AmCPV infection having higher survival (66.7%) and LC50 values (1000893.1796 polyhedra/ml) of AmCPV followed by Sarihan eco-race (50.7% survival and LC50 value of AmCPV 187203.6168 polyhedra/ml.) and Raily eco-race (25.3% survival and LC50 value of AmCPV 5176.37 polyhedra/ml.). Difference in total haemocyte count *i.e.* higher in tolerant (Daba) and lower in susceptible (Raily) eco-race may be in response to difference to their susceptibility to AmCPV infection.

Keywords: *Antheraea mylitta*, Am CPV, Haemocytes, Tolerance.

INTRODUCTION
In general, the silkworm breeds or the breeds of different geographical origin do differ in the degree of susceptibility to pathogens. Singh *et al* (2003) reported the difference in susceptibility of Indian silkworm breeds to Aspergillus infection. Aratake (1973) observed the strain difference of the silkworm, *Bombyx mori* L. in the resistance to a Nuclear Polyhedrosis Virus. Uzigawa and Aruga (1966) and Watanabe (1967) found the difference in survival of different silkworm strains fed with infectious flacherie virus and Cytoplasmic Polyhedrosis Virus respectively. Ratnsen *et al.*, (1999) reported that silkworm breed g133 exhibited three times higher survival than breed KA on challenge with the same dose of Nuclear Polyhedrosis Virus. Singh *et al* (2003) found that the tolerance of silkworm breed SU12 against Densonucleosis Virus was five times more than Zhenon1.

Insects show defense response through cellular and humoral components (Gupta, 1986). Humoral reactions involve slow synthesis of antibacterial and antiviral principles and require several hours for full expression. Cellular responses are direct interactions between circulatory haemocytes and invading non-self materials. The interaction is immediate and includes phagocytosis, nodulation and encapsulation (Gupta, 1986). In insects, several types of haemocytes are observed in the haemolymph (Jonesh, 1962 and Butt and Shields, 1996). Balavenkatasubbaiah *et al* (2001) recorded a significant difference in the total haemocyte counts of Nuclear Polyhedrosis Virus in the tolerant and susceptible silkworm breeds. The total haemocyte counts may indicate the susceptibility status of insect.

Tropical tasar silkworm, *Antheraea mylitta* Drury, an economically important insect is reared in out door condition on *Terminalia arjuna, Terminalia tomentosa* and *Shorea robusta* food plants to produce natural silk. Virosis caused by *Antheraea mylitta* Cytoplasmic Polyhedrosis Virus (AmCPV) is one of the important diseases, which cause 25-30% loss in cocoon crop (Sahay *et al,* 2000). The tasar silkworm eco-races viz. Daba, Sarihan and Raily, reared by tasar farmers in different parts of India are known for their good commercial characters (Cocoon weight, Shell weight, Silk Ratio *etc*.). Informations on susceptibility of the tasar silkworm eco-races to Cytoplasmic Polyhedrosis Virus infection are scanty. Hence, in the present study the susceptibility of these eco-races to Cytoplasmic Polyhedrosis Virus was tested based on survival rate and total haemocyte counts.

MATERIALS AND METHODS

Silkworm Eco-races

Three tasar silkworm eco-races namely Daba, Sarihan and Raily, reared by tasar farmers in different parts of India, known for their good commercial characters (Cocoon weight, Shell weight, Silk Ratio *etc*.) were selected for testing their susceptibility status to Cytoplasmic Polyhedrosis Virus infection.

Antheraea mylitta Cytoplasmic Polyhedrosis Virus (AmCPV) inoculum

Fresh Cytoplasmic Polyhedrosis Virus inoculum's was prepared from diseased silkworm. Completely whitened mid-gut obtained from cytoplasmic polyhedrosis silkworm at an advanced stage of infection was homogenized in sterile distilled water. The polyhedral suspension was filtered through a cheese – cloth, the filtrate was centrifuged at 3000 rpm for 15 minutes.The polyhedra were purified following Aizawa (1971) by repeated and differential centrifugation. The resultant pellet suspended in distilled water was examined under light microscope for purity. The density of polyhedra/ml was determined using haemocytometer. The polyhedral suspension in sterile distilled water was prepared to contain 1 x 10^7 polyhedra/ml. The desired concentrations of polyhedra suspension were prepared by serial dilution in distilled water.

Estimation of haemocytes count

Total haemocyte counts (THC) estimation in the haemolymph of all three silkworm breeds in late 3rd, 4th and 5th instar larva was determined following the method described by Tauber and Yeager (1935) and Jonesh (1962) using haemocytometer. Six larvae were collected from each replication, the haemolymph from all the 6 larvae was collected into two eppendoff tubes (3 larval haemolymph/tube) on ice and stored at 4°C. A total of 6 tubes represented 3 replication collections.

Inoculation of POBs of AmCPV and rearing of silkworm larvae

The silkworm larvae of all three eco-races were inoculated orally with 200 µl of AmCPV suspension containing 1 x 10^3,1 x 10^4, 1 x 10^5, 1 x 10^6, 1 x 10^7 polyhedra/ml by feeding of CPV smeared leaf 24 hrs after 2nd moult. Three replications with 100 silkworm larvae each were maintained separately in each eco-race for different concentration. A separate normal batch of 100 larvae of each breed was also maintained without virus inoculation for the estimation of total haemocyte counts. Silkworm larvae were reared on leaf of its primary food plant, *Terminalia tomentosa* (Assan) in indoor under normal rearing conditions up to spinning.

The observations were made on development of disease symptoms, larval mortality and total haemocyte counts. The dead larvae, during rearing, were examined microscopically for presence of concerned pathogens. Data recorded for mortality due to concerned pathogen, total haemocyte counts were statistically analyzed using Completely Randomized Design (Snedecor and Cochron, 1995).

RESULTS AND DISCUSSION

Daba, Sarihan and Raily eco-races of tasar silkworm are the choice of the rearers and reelers in different parts of India as the commercial value of these eco-races is more or less same. The Larva and cocoon characters of these eco-races are presented in table 1. The average Cocoon weight was 9.64

to 10.69g, Shell weight 1.27 to 1.51g and Silk Ratio 13.91 to 14.51 per cent.

The survival per cent of the tasar silkweorm eco-races is presented in figure 1. The survival of Daba, Sarihan and Raily was significantly different ($p < 0.05$) when challenged with the same concentration (1×10^5) of AmCPV. The higher survival (66.70%) of Daba showed their higher tolerance to AmCPV followed by Sarihan eco-race (50.7% survival) and Raily eco-race (25.30 % survival). The tolerance level of Daba and Sarihan eco-races against AmCPV was more than two times when compared with Railly eco-race.

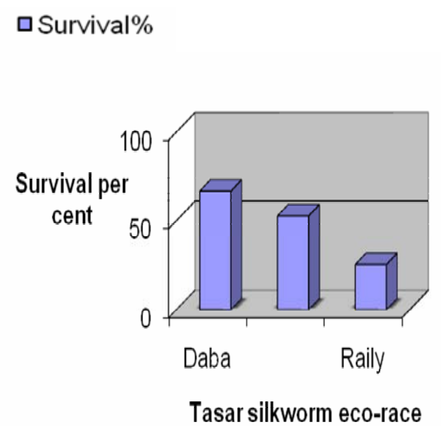

Fig 1. Survival per cent of different silkworm breeds infected with AmCPV (1×10^5 polyhedra/ml).

LC50 value of AmCPV to Daba, Sarihan and Raily eco-races are presented in table 2. Observation revealed that Daba eco-race was more tolerant to AmCPV infection followed by Sarihan and Raily. The LC50 value of AmCPV to Daba eco-race was higher (1000893.1796 polyhedra/ml) which decreeased to 187203.6168 polyhedra/ml in case of Sarihan eco-race and 5176.3714 polyhedra /ml in Raily eco-race. The results of total haemocyte counts (THC) estimated for different eco-races of tasar silkworm are presented in table 3. The THC was increased as the age of the larva increased from 3rd to 5th instar in all three eco-races. The total haemocyte counts in 3rd, 4th and 5th instar showed significant differences between the eco-races. The THC was least in Raily (11309.66±702.03, 15127.00±688.68 and 20156.67±764.28 in 3rd , 4th and 5th instar respectively). It was highest 14703.00 ±577.23, 18116.00±627.01 and 25461.00±722.45 during 3rd, 4th and 5th instar respectively in Daba.

Table 1. Characteristics of eco-races of *Antheraea mylitta* (Average of three generations: Mean ± SD.

Eco-race	Fecundity	Hatching %	Larval weight(g)		Cocoon harvest/ dfl	Female			Male			Average of female and male		
			Female	Male		CW (g)	SW (g)	SR (%)	CW (g)	SW (g)	SR (%)	CW (g)	SW (g)	SR (%)
Daba	337.00±16.00	64.62±6.79	39.56±5.01	25.09±2.03	56.46±6.56	12.20±0.70	1.67±0.38	13.70±2.46	9.26±0.47	1.37±0.32	14.70±2.80	10.69±0.54	1.51±0.34	13.97±2.90
Sarihan	206.06±5.35	60.55±8.33	34.92±4.70	23.92±3.44	34.40±12.00	11.34±0.68	1.51±0.20	13.40±2.19	8.20±1.29	1.28±0.27	15.63±2.48	9.64±1.15	1.39±0.22	14.51±1.12
Raily	272.2±30.72	54.17±5.20	36.65±2.72	22.97±1.54	33.06±3.91	10.50±1.74	1.36±0.01	13.23±1.95	9.21±2.95	1.18±0.005	15.12±1.82	9.96±1.02	1.27±0.01	13.91±1.07

CW= Cocoon weight., SW= Shell weight., SR= Silk Ratio.

Table 2. Probit analysis of the mortality % in response to AmCPV (1 x10^3 to 1 x 10^7).

Silkworm Eco-race	LC50	Y Mean	X Mean	Probit equation	Chi square	SE of b	Probability	Fiducial limit
Daba BV	1000893.1796	4.6664	5.3200	y= 2.0581 +0.4902902	2.7339	0.0484	0.4345	520554.1215 to 1924463.0205
Sarihan	187203.6168	4.9108	5.0782	y= 2.55777 +0.4594415 x	0.7407	0.0453	0.8636	102740.3217 to 341104.5783
Raily	5176.3714	5.4305	4.6118	y= 3.2191 +0.4795092 x	0.7214	0.0494	0.8682	2489.7046 to 10762.2490

Table 3. Total haemocyte counts in different silkworm eco-races.

Silkworm ecora-ces	3rd instar	4th instar	5th instar
Daba BV	14703.00±577.33	18116.00±627.01	25461.00±722.45
Sarihan	12276.67±932.75	16030.33±520.11	23533.00±649.28
Raily	11309.66±702.03	15127.00±688.65	20156.67±764.28
CD at 5%	1399.82	3845.87	2442.10

The results of survival per cent and LC50 values of AmCPV inferred that the tasar silkworm eco-races tested in the present study have varied degree of susceptibility to the infection of Cytoplasmic Polyhedrosis Virus. The Daba eco-race was found more tolerant to the AmCPV infection, which is followed by Sarihan and Raily. These results are corroborative with the findings of Uzigawa and Aruga (1966), Aratake (1973), Ratnasen *et al* (1999) and Singh *et al* (2003). This is the first report with the regard to the tolerance level of Indian tropical tasar silkworm eco-races to *Antheraea mylitta* Cytoplasmic Polyhedrosis Virus.

In the present study, Daba eco-race exhibited tolerance to CPV infection having significantly higher survival (66.70%) and high LC50 (1000893.1796 polyhedra/ml) of CPV possessed the higher number of THC also in 3rd, 4th and 5th instar larvae. Low survival of Raily and correspondingly low LC50 of CPV and THC shows its susceptibility to CPV infection. The observations of high THC in the tolerant silkworm eco-race point to the possibility of a correlation between the tolerance of the eco-race to disease and THC. Similar observations have been made in silkworm, *Bombyx mori* against fungal infection (Kawakami, 1965), *Periplanata americana* (Ennesser and Nappi, 1984) and arthropods (Gupta and Han, 1988). It is evident from the results that the haemocytes play an important role in defense against infection. In tolerant eco-race, the THC count is higher than that in susceptible eco-race. The findings of the present study may help in the breeding of disease resistance silkworm variety.

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