EFFECT OF CYTOPLASMIC POLYHEDROSIS ON SILKWORM (*BOMBYX MORI* L.) AND ITS TRANSMISSION FROM GENERATION TO GENERATION

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(Received : June, 7th, 2000)

ABSTRACT

The LC_{50} for larval mortality during fifth instar was 0.096×10^6 cytoplasmic polyhedral inclusion bodies ml^{-1} in Pure Mysore and 0.218×10^6 PIBs ml^{-1} in NB_4D_2 silkworms. Besides mortality of silkworms, there was a significant reduction in the cocoon weight, shell weight and shell ratio in the surviving silkworms. The F_1 progeny obtained by selfing the moths emerged from the silkworms inoculated with B*m*CPV exhibited inferiority in fecundity, hatchability, larval weight, cocoon yield, cocoon weight, shell weight, shell ratio, filament length,denier and effective rate of rearing, when compared to control batches.

Key words : *Bombyx mori*, **B*m*CPV, commercial characters, polyhedrosis.**

INTRODUCTION

The silkworm cocoon crops are highly unpredictable due to several factors including various diseases. Of various pathogens, the cytoplasmic polyhedrosis virus (B*m*CPV) of *Bombyx mori* L. is one of the most widely distributed viruses in the sericultural belt of Karnataka state and causes considerable damage to silk production. In Karnataka alone the infection of B*m*CPV accounted 27.76% (Samson *et al.*, 1988). In contrast to pebrine (*Nosema bombycis* Nageli), there is no clear-cut evidence about the mode of transmission of B*m*CPV from generation to generation, or about its effects on the commercial characters of the progeny. However, Hukuhara (1962) and Aruga and Nagashima (1962) reported that the B*m*CPV is transmitted from generation to generation in *Bombyx mori*. In contrast, surface contamination of eggs in *Pectionophora gossypiella* (Bullock *et al.*, 1969), *Heliothis virescens* (Sikorowski *et al.*, 1973 Merry and Dulmage, 1975) is an important means of transmitting the CPV from generatin to generation. Neilson (1965) in four species of lepidoptera, Vail *et al.*, (1969) and, Vail and Gouch (1970) in *Trichoplusiani*, Bullock *et al.*, (1970) in *Pectinophora gossipiella*, Magnoler (1974) in *Parthetria dispar*, Simmons and Sikorowski (1973) and Sikorowski and Thomson (1979) in *Heliothis virescens*, observed the larval mortality, increased larval and pupal duration, reduced larval and pupal weight, reduced moth emergence with deformed, undersized adults, reduced fecundity in the diseased individuals. But, studies on the effects of B*m*CPV on commercial characters of silkworm *Bombyx mori* and its transmission from generation to generation are rather scarce. Therefore, present investigation was carried out.

MATERIALS AND METHODS

Two mulberry silkworm races namely Pure Mysore (multivoltine), and NB_4D_2 (bivoltine) at the age of fifth instar first day and a stock of cytoplasmic polyhedrosis virus (B*m*CPV) were used for the study. The cytoplasmic polyhedral inclusion bodies (PIBs), obtained from Sericulture Department, University of Agricultural Sciences, G.K.V.K., Bangalore, India, were *per orally* inoculated into the silkworm larvae for multiplication. Isolation and purification of PIBs were carried out by following the method described by Balakrishnappa and Honnaiah (1992).

The silkworm rearing was conducted in the laboratory following the method described by Krishnaswami (1978). For determination of breed susceptibility, a total of seven batches, each batch containing 50 worms in triplicate, were inoculated by oral injection with 40µl of different concentratins of PIBs viz., 6.25×10^6, 3.125×10^6, 1.562×10^6, 0.781×10^6, 0.391×10^6, 0.195×10^6 and 0.0976×10^6 ml^{-1} polyhedral bodies in 0.75% NaCl solution. The control worms received the same amount of 0.75% NaCl solution only. Later, the worms were allowed to complete larval stage, spinning, pupation and moth emergence. Number of cocoons harvested from each batch were considered for calculation of the breed susceptibility. The moths emerged from B*m*CPV inoculated larvae were selfed and the F_1 progeny raised were used to study the effects of B*m*CPV on the economic characters of silkworm. Two doses of B*m*CPV, viz., 1.562×10^6 ml^{-1} (T_1) and 3.125×10^6 ml^{-1} (T_2) were selected for inoculation after preliminary studies on breed susceptibility. The larvae were allowed to complete larval duration, spinning, pupation and moth emergence. Cocoons were harvested, preserved racewise and treatmentwise at room temperature of 25±2°C and relative humidity of 75± 5%. The procedure followed for the preparation of layings & incubation, silkworm rearing and assessment of economic traits are as described by Narasimhanna (1988), Krishnaswami (1978) and Mahesha (1997), respectively. The data derived from the above, experiments were statistically analysed by one way ANOVA (Fisher and Yates, 1953) and Duncan Multiple Range Test (Duncan, 1955). The LC_{50} values were calculated for B*m*CPV infection by using probit analysis (Fenney, 1971).

RESULTS AND DISCUSSION

The LC_{50} for larval mortality during fifth instar was $0.096 \pm 0.0024 \times 10^6$ PIBs ml^{-1} in Pure Mysore followed by NB_4D_2 ($0.28 \pm 0.0027 \times 10^6$ PIBs ml^{-1}). This indicates that Pure Mysore was more sensitive to B*m*CPV when compared with NB_4D_2 race. In Pure Mysore as well as NB_4D_2 races, the viability rate was higher in the control batches and gradual reduction was noticed as the dose of B*m*CPV increased. According to Bhaskar (1996), the Pure Mysore is sensitive to B*m*CPV than NB_4D_2 in early stages of larval development. However, contrary to the general trend, the multivoltine Pure Mysore, was found to be more sensitive to B*m*CPV as compared to a bivoltine NB_4D_2 race. Such a contradiction might have direct relationship to the body weight as well as larval duration; that is, lighter race with longer larval duration (Pure Mysore) is more susceptible to B*m*CPV as against the heavier race with lesser larval duration (NB_4D_2). Apart from the mortality of silkworms, there was a significant reduction in the cocoon weight, shell weight and shell ratio in the surviving silkworms of both Pure Mysore as well as NB_4D_2 races (Table 1). This might be due to reduced feeding accompanied by the reduced

Table 1. Effect of cytoplasmic polyhedrosis on larval mortality and cocoon charecters

Dose of Bm CPV	Race: Pure Mysore No. of cocoon harvested	Pure Mysore Cocoon weight (g)	Pure Mysore Shell weight (g)	Pure Mysore Shell ratio (%)	Race: NB_4D_2 No. of cocoons harvested	NB_4D_2 Cocoon weight (g)	NB_4D_2 Shell weight (g)	NB_4D_2 Shell ratio (%)
C_0 (Control)	46	0.958	0.139	14.51	45	1.884	0.364	19.32
C_1 ($0.098 \times 10^6 ml^{-1}$)	22	0.850	0.102	12.00	26	1.804	0.301	16.68
C_2 ($0.195 \times 10^6 ml^{-1}$)	18	0.840	0.087	10.36	19	1.786	0.287	15.79
C_3 ($0.391 \times 10^6 ml^{-1}$)	13	0.821	0.081	9.87	15	1.754	0.265	15.11
C_4 ($0.781 \times 10^6 ml^{-1}$)	12	0.778	0.074	9.51	14	1.702	0.256	15.04
C_5 ($0.562 \times 10^6 ml^{-1}$)	10	0.750	0.070	9.33	12	1.671	0.251	14.96
C_6 ($3.125 \times 10^6 ml^{-1}$)	7	0.733	0.069	9.41	9	1.639	0.243	14.82
C_7 ($6.25 \times 10^6 ml^{-1}$)	5	0.701	0.062	9.41	6	1.570	0.226	14.39
F Ratio	177.461	133.480	12.055	5.437	12.384	212.728	400.288	10.121
Probability	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000

digestibility and absorption, since the pathogen destroys the midgut tissue. Moreover, the food material absorbed by the pathogen might be utilized for its reproduction and metabolism. In addition, the energy derived from the digested food must have distributed between the host as well as virus for their mutual defense purpose.

The F_1 progeny obtained by selfing the moths emerged from the silkworms inoculated with *Bm*CPV exhibited inferiority as compared to control batches in almost all the economic characters analysed. The size of the eggs in the silkworm batches treated with *Bm*CPV was small in Pure Mysore, but NB_4D_2 did not show significant reduction in the egg size. Almost all commercial characters like fecundity, hatchability, larval weight, cocoon yield, cocoon weight, shell weight and ratio, filament length, denier and effective rate of rearing showed reduction when compared to the control sets of both the races (Table 2 and 3). Vail *et al.* (1969) and, Vail and Gouch (1970) in Trichoplusia ni made similar observations. Bullock *et al.* (1970) in *Pectinophora gossipiella*, Simmons and Sikorowski (1973) and. Sikorowski and Thomson (1979) in *Heliothis virescens*. The reduction in the egg size and fecundity might be due to the deviaiton of digested food from normal metabolic state to synthesis of viral proteins and/or due to reduced rate of ingestion, digestion and assimilation of food owing to the malfunction of the midgut of the worms infected with *Bm*CPV. The inferiority in the economic traits of progeny might be due to the following reasons. Firstly, as the egg size was found to be smaller in the *Bm*CPV treated batches, the quantity of yolk, which is

Table 2. Effect of cytoplasmic polyhedrosis on commercial characters of F_1 progeny (Race : Pure Mysore)

Dose of BmCPV	Fecundity	Hatching (%)	Wt. of 10 V^{th} age larva (g)	Larval duration (h)	Yield per 10000 larvae brushed Number	Yield per 10000 larvae brushed Weight (kg)	Single cocoon wt. (g)	Single shell wt (g)	Shell ratio	Filament length (m)	Denier	ERR
Control (00)	504	98.49	21.52	672	9400	7.445	0.856	0.114	13.32	381	1.94*	94.00
T_1 ($1.562x10^6ml^{-1}$)	439	96.11	20.04	672	9324	6.748	0.784	0.101*	12.88*	348	1.92*	93.24*
T_2 ($3.125X10^6ml^{-1}$)	401	95.41	19.46	672	9309	6.595	0.771	0.096*	12.45*	321	1.92*	93.09*
F Ratio	42.39	97.55	114.89	-	37.783	235.93	265.73	9.15	3.10	55.67	2.40	7.17
Probability	0.000	0.000	0.000	-	0.000	0.000	0.000	0.015	0.119	0.000	0.172	0.026

Table 3. Effect of cytoplasmic polyhedrosis on commercial characters of F_1 progeny (Race : NB_4D_2)

Dose of BmCPV	Fecundity	Hatching (%)	Wt. of 10 V^{th} age larva (g)	Larval duration (h)	Yield per 10000 larvae brushed Number	Yield per 10000 larvae brushed Weight(kg)	Single cocoon wt. (g)	Single shell wt (g)	Shell ratio	Filament lenght (m)	Denier	ERR
Control (00)	580	98.11	44.05	576	9418	17.800	1.950	0.376	19.28	1054	2.28	94.18
T_1 ($1.562x10^6ml^{-1}$)	520*	95.46	41.71	576	9340	16.680	1.846	0.312	16.90*	924	2.21*	93.40
T_2 ($3.125X10^6ml^{-1}$)	496*	94.01	40.09	576	9311	15.955	1.774	0.284	16.00*	881	2.22*	93.11
F Ratio	25.880	34.215	36.238	-	162.124	431.008	403.008	31.609	12.143	16.638	21.500	271.784
Probability	0.001	0.001	0.001	-	0.000	0.000	0.000	0.001	0.008	0.004	0.002	0.000

The variation between control and experimental sets, and among different doses are all significant at 5% level.

* Not significant at 5% level.

reserved for embryonic development, was also reduced; ultimately weak larva may hatch out. Secondly. it is also possible that B*m*CPV can be transmitted from generation to generation in an occult state, which might render the larva of *Bombyx mori* weak and incapable to perform normal metabolism. Thus, inferior economic characters are produced. The findings of Hukuhara (1961) and Aruga and Nagashima (1962) support such reasoning. They reported that the B*m*CPV is transmitted from generation to generation in *Bombyx mori*. In contrast, Sikorowski *et al.* (1973) reported that CPV is transmitted in the tobacco budworm, *Heliothis virescens,* on the surface of the eggs rather than inside them. Bullock *et al.* (1969) also concluded that the surface contamination of eggs is an important means of transmitting the CPV of the pink bollworm, *Pectinophora gossypiella*. However, Sikorowski *et al.* (1971) observed that the virus particles are present in the haemolymph of *Heliothis virescens* larvae and in the adult. This result suggested that the developing eggs might be under the influence of haemolymph, which contains virus particles. Thus, inferior commercial characters might be due to weak larvae hatched out from the undersized eggs produced under the influence of B*m*CPV .

Hence, the present investigation clearly indicated that the progeny obtained by selfing the moths emerged from B*m*CPV inoculated silkworms, exhibited inferiority in almost all characters analysed, and this knowledge can be used in the sericulture industry during the selection of parent seed cocoons for the preparation of disease free layings either for commercial or academic purposes, which have a vital role in the improvement of the industry.

ACKNOWLEDGEMENT

Thank are due to University of Mysore, for extending the facilities to carry out this work.

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