Int. J. Indust. Entomol. Vol. 12, No. 2, 2006, pp. 95~100

International Journal of
Industrial Entomology

Screening of Promising Bivoltine Hybrids of Mulberry Silkworm for their Susceptibility to *Bombyx mori* Nuclear Polyhedrosis Virus and *Bombyx mori* Infectious Flacherie Virus

L. Hemanth Kumar*, Ratna Sen, B. Nataraju and M. Mamatha
Central Sericultural Research and Training Institute, Mysore-570008, India.

(Received 28 January 2006; Accepted 1 June 2006)

Central Sericultural Research and Training Institute, Mysore have evolved several highly productive bivoltine hybrids which can produce international grade raw silk. Among them CSR2 × CSR4, CSR2 × CSR5, CSR3 × CSR6, CSR17 × CSR16, CSR18 × CSR19 and CSR12 × CSR6 are being popularized in the field. There is a minimum difference in their economic characters but they appear to differ in survival. Though they are productive under high input management conditions, they are very susceptible to different diseases under normal rearing practices. No systematic attempts have been made to test their susceptibility status / resistance. Thus the present study is a modest attempt to screen the above six productive bivoltine hybrids to two important pathogens *viz.*, *Bombyx mori* Nuclear Polyhedrosis Virus (BmNPV) and *Bombyx mori* Infectious Flacherie Virus (BmIFV) along with existing hybrid, KA × NB4D2 to assess their susceptibility / resistance. The results shows that the productive hybrid CSR2 × CSR4 is the most resistant to BmNPV and it is suggested by its highest LC_{50} value followed by CSR12 × CSR6, KA × NB4D2, CSR3 × CSR6, CSR17 × CSR16, CSR18 × CSR19, CSR2 × CSR5. Based on the LC_{50} value and LT_{50} values for BmIFV , the hybrid KA × NB4D2 was found to be the most resistant (1st position) one followed by CSR3 × CSR6 (2nd position) CSR2 × CSR (3rd position) and CSR12 × CSR6 (4th position) CSR17 × CSR16, CSR18 × CSR19 (5th position) and CSR2 × CSR5 being the least. The response of 7 bivoltine hybrids to both the pathogens BmNPV and BmIFV indicates that, the hybrids CSR2 × CSR4, CSR12 × CSR6 and KA × NB4D2 were found to be the most resistant when compared to others. Further, KA × NB4D2 being less productive hybrid with a shell ratio of 20.08%, the other two hybrids CSR2 × CSR4 (Cocoon shell ratio, 21.44%) and CSR12 × CSR6 (cocoon shell ratio, 23.45%) can be considered to be most productive with superior quality cocoon and resistant to both BmNPV and BmIFV pathogens. The overall study indicated that the hybrid CSR2 × CSR5 is the most susceptible hybrid to both the pathogens.

Key words: Silkworm hybrids, *Bombyx mori*, susceptibility, BmNPV, BmIFV

Introduction

The domestication of mulberry silkworm for the past several thousands of years has rendered them most susceptible to diseases. The sources of pathogenic microorganisms are normally the diseased silkworms and the contaminated rearing environment. These infectious microbes cause secondary infection and spread diseases (Ishikawa and Miyajima, 1964) in silkworm rearing leading to cocoon crop loss. Survey reports from different sericultural areas in India have revealed that the cocoon crop loss is often due to diseases (Samson *et al.*, 1990; Savanurmath *et al.*, 1995; Sivaprakasam and Rabindra, 1995). Among them "Nuclear Polyhedrosis" caused by *Bombyx mori* Nuclear Polyhedrosis Virus (BmNPV) and "Infectious flacherie" caused by *Bombyx mori* Infectious Flacherie Virus (BmIFV) are common. The natural incidence of Nuclear polyhedrosis virus in silkworm is common during final instar. The severity of the disease is very high under conditions of contaminated rearing environment and incomplete or no disinfection and hygiene (Prasad, 1999).

*To whom correspondence should be addressed
Central Sericultural Research and Training Institute, Mysore-570008, India. Tel: +91-821-2362571; Fax: +91-821-2362845; E. mail: hemanthkumar_1979@rediffmail.com

Infectious flacherie caused by *B. mori* Infectious flacherie virus is another important viral disease. The pathogen along with *Streptococcus faecalis* is known to cause high incidence of flacherie in silkworm rearing. In Karnataka 22.37% of crops is known to suffer from the disease. The Pathogens are extruded into the rearing environment from the day 2 along with faeces and contaminate the rearing environment.

Generally bivoltine silkworms are comparatively more susceptible to BmNPV and BmIFV and Multivoltine hybrids and Multi × bivoltine hybrids are reported to be comparatively less susceptible (Baig *et al.*, 1991). Apart from this, there are many reports available in literature regarding susceptibility / tolerance of different races to various diseases of silkworm (Tanada, 1967; Liu Shi Xian, 1984; Samson, 1987; Chinnaswamy and Devaiah, 1984; Nataraju, 1995). In view of poor hygienic rearing conditions and incomplete disinfection practices prevailing largely at farmer's level, there is a need to rear silkworm hybrids, which have resistance or low level of susceptibility to a number of pathogens. Adoption of such hybrids will go a long way in minimizing the cocoon crop loss to a greater extent. Thus, an attempt has been made to know the susceptibility status of the popular bivoltine hybrids in the field to two important silkworm diseases such as BmIFV and BmNPV.

Materials and Methods

Seven F1 hybrids derived from the productive bivoltine silkworm parental stocks have been selected to study their susceptibility status to *B. mori* Nuclear Polyhedrosis Virus (BmNPV) and *Bombyx mori* Infectious Flacherie Virus (BmIFV). The selected bivoltine hybrids are: CSR 2 × CSR 4, CSR 2 × CSR 5, CSR 3 × CSR 6, CSR 17 × CSR16, CSR 18 × CSR 19, CSR 12 × CSR 6, KA × NB4D2.

Preparation of BmNPV inoculum

The Polyhedral inclusion bodies of BmNPV was extracted from the haemolymph of the infected fifth instar larvae and purified by the method of Govindan *et al.* (1998) with suitable modifications. Haemolymph was collected by cutting the pro legs of terminally infected larvae into a sterilized tube containing a few crystals of phenylthiourea. The haemolymph was stored in the refrigerator for one week. Then it was diluted two fold by sterilized distilled water containing Penicillin at the rate of 500-units/ml and Streptomycin sulphate at the rate of 500 mg/ml. It was mixed thoroughly and kept at 5°C over night. The haemolymph was filtered through cheesecloth and centrifuged at 5,000 rpm for 10 min. The pellet was re-suspended in half the quantity of the original volume of sterilized distilled water and centrifuged at 5,000 rpm for 10 min. The cycle of washing and centrifugation at 5,000 rpm was repeated twice. The suspension was checked under microscope for the complete absence of debris. The pellets after final centrifugation was suspended in a small quantity of distilled water and stored at 5°C until use. Before use the polyhedral inclusion bodies were counted in the suitably diluted suspension of the mother stock using haemocytometer under Leitz-diaplan microscope. The concentration is expressed as polyhedral inclusion bodies/ml (PIB/ml). The mother stock was diluted with sterile distilled water to obtain different concentrations at constant interval of dilution.

Preparation of BmIFV inoculum

The terminally infected larvae that were inoculated artificially with an infected dose of BmIFV were collected and the midgut was dissected out. The tissue was ground with sterilized distilled water and 10% homogenate was prepared. The homogenate was centrifuged at 5,000 rpm for 10 min. The supernatant collected was served as mother stock of BmIFV. Before use, the mother stock was diluted with sterilized distilled water to obtain desired concentration at a fixed interval of dilution.

Inoculation

Newly ecdysed third instar silkworm larvae, derived from the pooled population 8-10 disease free layings of each hybrid, were utilized for the experiment. The mother stock of both the pathogens for the determination of half lethal concentration (LC_{50}) and half lethal time (LT_{50}) are shown in Table 1.

Hundred newly ecdysed 3rd instar larvae, kept for each concentration, were fed once with 1 ml of inoculum of the respective pathogens. A population with equal number of larvae treated only with distilled water was maintained as control for Abbots correction. The larvae were fed thrice with fresh mulberry leaves of V1 variety and reared as per the standard rearing. The symptoms of both the diseases (Nuclear polyhedrosis and Infectious flacherie) were observed carefully and the number of diseased larvae was

Table 1. Different concentrations of stock inoculum of BmNPV and BmIFV

Pathogen	Conc. 1	Conc. 2	Conc. 3	Conc. 4
BmNPV (PIB/ml)	8×10^5	4×10^6	2×10^7	1×10^8
BmIFV (Dilution)	10^{-12}	10^{-10}	10^{-8}	10^{-6}

recorded daily up to 10 days in case of BmNPV and 15 days in case of BmIFV. The experiment was repeated 3 times.

Statistical analysis

The mean and the standard deviation were calculated for cumulative mortality from the data obtained in 3 consecutive trials of the experiment. Log-concentration - Probit analysis was done by least square method to determine the LC_{50} for BmNPV and LC_{50} as well as LT_{50} for BmIFV by utilizing computer software Indostat.

Rearing and reeling performance

In a separate set of experiment all the hybrids were reared in mass up to 2nd instar and there after 300 larvae from each hybrid were retained till cocooning to study their rearing and reeling performance under prevailing room temperature (25°C and relative humidity 60 ± 5). The cocoons were harvested on 5/6th day of mounting and cocoon assessment has done with 25 male and 25 female cocoons. 100 cocoons from each hybrid were selected for test reeling and reeled on multiends reeling machine to know their reeling performance.

Results

Response to BmNPV

Table 2 shows the cumulative mortality of seven bivoltine hybrids in response to four concentrations of BmNPV. Out of 100 larvae inoculated each with four concentrations of BmNPV, the least number of larvae infected was 20 in the lowest concentration (8×10^5 PIB/ml), whereas the same was 80.67 in the highest concentration (1×10^8). In each hybrid, the mortality increased with the increase in the concentration of BmNPV.

The average LC_{50} value was found to be highest in the hybrid CSR2 × CSR4 (3.22×10^7 PIB/ml) followed by CSR12 × CSR6 (2.22×10^7 PIB/ml), KA × NB4D2 (1.61×10^7 PIB/ml), CSR3 × CSR6 (1.35×10^7 PIB/ml), CSR17 × CSR16 (1.09×10^7 PIB/ml), CSR18 × CSR19 (9.08×10^6 PIB/ml) and the least in CSR2 × CSR5 (8.5×10^6 PIB/ml).

The resistance ratio calculated between CSR2 × CSR4 which has the highest LC_{50} value (Table 6) and the rest of the hybrids showed a range from the highest of 3.795 with CSR2 × CSR5 followed by 3.552 with CSR18 × CSR19, 2.940 with CSR17 × CSR16, 2.393 with CSR3 × CSR6,

Table 2. Cumulative mortality and LC_{50} in 7 bivoltine hybrids of silkworm inoculated with different concentrations of BmNPV (mean of 3 trials ± S.D.)

Hybrid	% Mortality due to different concentrations of BmNPV polyhdra/ml				LC_{50}
	8.0×10^5	4.0×10^6	2.0×10^7	1.0×10^8	
CSR2 × CSR4	25.666 ± 6.429	33.666 ± 8.083	40.333 ± 10.214	66.333 ± 6.027	3.22×10^7
CSR2 × CSR5	36.333 ± 4.041	40.666 ± 2.309	64.666 ± 9.291	68.666 ± 13.316	8.50×10^6
CSR3 × CSR6	22333 ± 7.023	32.000 ± 5.291	46.666 ± 9.291	80.666 ± 3.786	1.35×10^7
CSR17 × CSR16	25.333 ± 11.051	30.333 ± 16.010	53.333 ± 2.081	78.000 ± 13.453	1.09×10^7
CSR18 × CSR19	23.666 ± 11.930	36.333 ± 9.073	60.666 ± 7.505	80.333 ± 10.503	9.08×10^6
CSR12 × CSR6	20.000 ± 7.549	27.333 ± 3.055	47.333 ± 8.144	80.000 ± 16.462	2.22×10^7
KA × NB4D2	24.333 ± 7.925	32.333 ± 19.655	50.333 ± 14.571	77.666 ± 14.571	1.61×10^7

Table 3. Cumulative mortality and LC_{50} in response to 4 concentrations of BmIFV in 7 bivoltine hybrids (Mean of 3 trials ± S.D.)

Hybrid	% Mortality due to different dilutions of BmIFV				LC_{50}
	10^{-12}	10^{-10}	10^{-8}	10^{-6}	
CSR2 × CSR4	54.333 ± 11.151	68.666 ± 4.041	74.000 ± 8.717	91.000 ± 8.544	$10^{-13.23}$
CSR2 × CSR5	52.333 ± 12.220	65.333 ± 8.505	83.000 ± 2.000	89.666 ± 9.291	$10^{-15.22}$
CSR3 × CSR6	61.666 ± 5.859	75.666 ± 11.150	78.000 ± 7.549	94.666 ± 2.309	$10^{-12.24}$
CSR17 × CSR16	57.666 ± 4.509	65.333 ± 3.0555	85.333 ± 3.786	95.666 ± 33.518	$10^{-13.11}$
CSR18 × CSR19	54.666 ± 4.041	70.666 ± 5.033	84.666 ± 6.110	87.333 ± 5.859	$10^{-12.16}$
CSR12 × CSR6	56.666 ± 14.047	74.000 ± 10.535	89.000 ± 10.000	99.666 ± 0.577	$10^{-13.13}$
KA × NB4D2	53.333 ± 12.342	70.333 ± 9.504	83.000 ± 11.135	85.666 ± 9.609	$10^{-12.16}$

2.004 with KA × NB4D2 and the least of 1.445 (least) with CSR12 × CSR6.

Response to BmIFV

Table 3 shows the cumulative mortality of seven bivoltine hybrids in response to four concentrations of BmIFV. Out of 100 larvae inoculated each with four concentrations of BmIFV, the least number of infected larvae was 52.333 in the lowest concentration (10^{-12} dilution) whereas the same was 99.666 in the highest concentration (10^{-6} dilution).

The LC_{50} value was found to be highest in the hybrid KA × NB4D2 ($10^{-12.16}$ dilution) followed by CSR18 × CSR19 ($10^{-12.16}$ dilution), CSR3 × CSR6 ($10^{-12.24}$ dilution), CSR17 × CSR16 ($10^{-13.11}$ dilution), CSR12 × CSR6 ($10^{-13.13}$ dilution), CSR2 × CSR4 ($10^{-13.23}$ dilution) and the least in case of CSR2 × CSR5 ($10^{-15.22}$ dilution).

Since the infection with BmIFV is a chronic phenomenon, the LT_{50} values (days post inoculation) for each concentration of BmIFV in seven hybrids were also determined and the data is presented in (Table 4). Since a variability was observed in four concentrations inoculated to the hybrids, the LT_{50} at a particular concentration of BmIFV in each hybrid was given scores depending upon their values which is presented in (Table 5) .The total score (sum of the scores allotted to each concentration) of the hybrid KA × NB4D2 was found to be highest with 25 scores followed by CSR3 × CSR6 with 18 scores, CSR2 × CSR4 and CSR12 × CSR6 each with 16 scores, CSR17 × CSR16 and CSR18 × CSR19 with 15 scores each and the lowest being CSR2 × CSR5 with 12 scores. The rearing and reeling performance of seven hybrids is presented in Table 7 and 8. The rearing results indicated that under normal rearing conditions, the hybrid CSR12 × CSR6 have shown highest pupation rate of 99.2% with a shell ratio of 23.4%; where as CSR2 × CSR4 has shown 93.2% pupation with a shell ratio of 21.4% showing their superiority (Table 7). The reeling results indicated longer filament length of 1245 mts in CSR3 xCSR6 followed by CSR17 × CSR16 with 1188 mts (Table 8). However top two hybrids CSR2 × CSR4 and CSR12 × CSR6 have shown a filament length of 1075 and 1173 meters respectively.

Table 6. Resistance ratio between the CSR2x CSR4 and rest of the hybrids (grading for BmNPV)

Hybrid	Resistance ratio	Grading
CSR2 × CSR4	-	I
CSR12 × CSR6	1.455	II
KA × NB4D2	2.004	III
CSR3 × CSR6	2.393	IV
CSR17 × CSR16	2.940	V
CSR18 × CSR19	3.552	VI
CSR2 × CSR5	3.795	VII

Table 4. LT_{50} in response to 4 concentrations of BmIFV in 7 bivoltine hybrids

Hybrid	LT_{50} values (Days post inoculation) of bivoltine hybrids in different dilutions of BmIFV			
	10^{-12}	10^{-10}	10^{-8}	10^{-6}
CSR2 × CSR4	13.37	11.02	10.67	9.462
CSR2 × CSR5	12.48	11.43	10.09	9.251
CSR3 × CSR6	12.91	10.22	10.30	9.687
CSR17 × CSR16	12.03	11.54	10.35	9.193
CSR18 × CSR19	12.85	11.19	10.05	10.270
CSR12 × CSR6	12.83	11.20	10.67	9.642
KA × NB4D2	13.75	11.28	11.12	10.180

Table 5. Scores alloted to 7 bivoltine hybrids based on their LT_{50} to 4 concentrations of Bm IFV

Hybrid	10^{-12}	10^{-10}	10^{-8}	10^{-6}	Total score	Grade
KA × NB4D2	7	5	7	6	25	I
CSR3 × CSR6	5	4	4	5	18	II
CSR2x CSR4	6	1	6	3	16	III
CSR12 × CSR6	3	3	6	4	16	III
CSR18 × CSR19	4	2	2	7	15	IV
CSR17 × CSR16	1	7	5	2	15	IV
CSR2 × CSR5	2	6	3	1	12	V

Table 7. Rearing Performance of 7 popular bivoltine hybrids

Hybrid	Hatching (%)	TLD (Days: hrs)	wt. of 10 mature larvae (g)	Pupation rate (%)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
CSR2 × CSR4	97.8	21:12	52.0	93.2	2.021	0.435	21.4
CSR2 × CSR5	95.7	22:00	51.2	90.0	2.082	0.440	21.3
CSR3 × CSR6	95.7	22:00	54.1	93.6	2.008	0.480	23.9
CSR17 × CSR16	92.4	21:12	50.4	96.8	1.899	0.421	22.2
CSR18 × CSR19	96.7	21:12	43.5	97.2	1.723	0.369	21.4
CSR12 × CSR6	91.1	21:12	53.2	99.2	1.865	0.437	23.4
KA × NB4D2	97.4	21:12	47.0	77.6	1.845	0.370	20.0

Table 8. Reeling performance of 7 popular bivoltine hybrids

Hybrid	Filament length (m)	Denier (d)	Renditta (kg)	Raw silk (%)	Raw silk (%)	Reel. (%)	Neat. (p)
CSR2 × CSR4	1075	2.90	5.82	17.1	83.9	83.05	93
CSR2 × CSR5	1054	2.99	5.93	16.8	79.0	85.45	93
CSR3 × CSR6	1245	2.80	5.17	19.3	80.7	79.03	94
CSR17 × CSR1	1188	2.68	5.36	18.6	83.7	84.21	93
CSR18 × CSR1	980	2.81	5.62	17.7	82.8	81.66	93
CSR12 × CSR6	1173	267	5.34	18.7	79.7	74.13	93
KA × NB4D2	1012	2.72	6.02	16.7	82.6	90.74	92

Discussion

There are many silkworm varieties or strains whose genetic constitutions are almost homozygous, and they could be studied intensively to establish the basis for genetic resistance to viral diseases. In the present investigation, highest LC_{50} value (3.22×10^7 PIB/ml) of the hybrid CSR2 × CSR4 indicates that this hybrid has the maximum resistance to BmNPV as compared to other hybrids. The least LC_{50} (8.5×10^6 PIB/ml) of the hybrid CSR2 × CSR5 indicates that this hybrid is the most susceptible among the hybrids, tested. The rest of the hybrid position lies in between these two extremes.

The least resistance ratio of 1.455 between CSR2 × CSR4 and CSR12 × CSR6 is suggestive of the least difference in the resistance to BmNPV between CSR2 × CSR4 and CSR12 × CSR6 as compared to other hybrids whereas the highest resistance ratio of 3.795 between CSR2 × CSR4 and CSR2 × CSR5 is suggestive of the maximum difference in resistance between these two hybrids as compared to other hybrids. Depending upon the resistance ratio between CSR2 × CSR4 which has exhibited the highest resistance, and the rest of the hybrids, a grading can be observed among them (Table 6). The position of the hybrid CSR2 × CSR4 is I followed by CSR12 × CSR6 as II, KA × NB4D2 as III, CSR3 × CSR6 as IV, CSR17 × CSR16 as V, CSR18 × CSR19 as VI and CSR2 × CSR5 as VII.

The highest LC_{50} value of $10^{-12.16}$ (dilution) of the hybrid KA × NB4D2 indicates that this hybrid has the maximum resistance to BmIFV as compared to other hybrids. The LC_{50} value of the hybrid CSR2 × CSR5 ($10^{-15.22}$ dilution) indicates that this hybrid is the most susceptible among the hybrids tested. Rests of the hybrids are in between these two extremes. The LT_{50} value of these hybrids follows more or less the same gradation as that of LC_{50} .The total scores allotted to the hybrid KA × NB4D2 is 25 which is the highest among all the hybrids suggesting its highest position in its resistance to BmIFV. Other hybrids are far behind from KA × NB4D2 as can be seen from the total scores received by them (Table 5). The least score of 12 received by the hybrid CSR2 × CSR5 is suggestive of its highest susceptibility to BmIFV.

Depending upon the total scores (Table 5) received by the seven hybrids, the position of KA × NB4D2 is I followed by CSR3 × CSR6 as II, CSR2 × CSR4 CSR12 × CSR6 as III, CSR17 × CSR16 and CSR18 × CSR19 as IV and CSR2 × CSR5 as V.

As seen from the response of seven bivoltine hybrids to BmNPV and also BmIFV, three hybrids *viz.* CSR2 × CSR4, KA × NB4D2 and CSR12 × CSR6 are important which have been graded in I to III position is suggestive of their higher resistance to BmNPV as well as BmIFV. Although KA × NB4D2 has shown greater resistance to BmNPV and BmIFV, this hybrid is less productive in terms of its economic characters (Tables 7 and 8) as compared to other two hybrids *viz.* CSR2 × CSR4 and CSR12 × CSR6. Aratake (1973a, b) also reported large differences in susceptibility of silkworm breeds to BmNPV and BmIFV among different breeds of silkworm. Both the hybrids CSR2 × CSR4 and CSR12 × CSR6 have exhibited greater resistance to BmNPV and BmIFV. A comparative look of their economic characters (Tables 7 and 8) shows that the hybrid CSR12 × CSR6 has an edge over CSR2 × CSR4 in its economic characters like cocoon-shell ratio (23.45%), average filament length (1173 meters), denier (2.67), renditta (5.34) and raw silk content (18.70%) although raw silk recovery is comparatively less (79.70%) probably due to low reelability (74.13%), as compared to CSR2 × CSR4 where the same is 83.99%. In the present investigation, popular hybrids are selected for study to provide basic information to the farmers to remove their apprehensions about a particular hybrids choice / performance in the field. Generally hybrids are homozygous and resistant to adverse climatic conditions. The two major disease chosen were genetically governed by polygenes and the rearing environment will modify it. If they are genetically resistant/tolerant to certain extent, more cocoon yield can be expected from such hybrids.

Thus, it could be concluded that the productive hybrids CSR12 × CSR6 and CSR2 × CSR4 are the best hybrids on account of their higher resistance to important pathogens like BmNPV causing grasserie and BmIFV causing viral flacherie and can be more preferred by the farmers.

References

Anonymous (1994) Studies on the prevalence of non-occluded viral diseases of silkworm. Annual Report, Mysore. pp.91.

Aratake, Y. (1973a) Difference in the resistance to infectious flacherie virus between the strains of silkworm *Bombyx mori* L. *Sansi Kenkyu* **86**, 48-57.

Aratake, Y. (1973b) Strain difference of the silkworm, *Bombyx mori* L. in the resistance to a nuclear polyhedrosis virus. *J. Seric. Sci. Jpn.* **42**, 230-238.

Baig, M., S. D. Sharma, M. Balavenkatasubbaiah, M. V. Samson, T. O. Shashidharan and M. K. R. Noamani (1991) Relative susceptibility of different races of silkworm, *Bombyx mori* L. to nuclear polyhedrosis under natural and induced conditions. *Sericologi.* **31**, 417-420.

Chinnaswamy, K. P. and M. C. Devaiah. (1984) Susceptibility of different races of silkworm, *Bombyx mori* L. to Aspergillosis caused by *Aspergillus tamarii* Kata. *Sericologia* **24**, 513-517.

Govindan, R., T. K. Narayanaswamy and M. C. Devaiah (1998) Viral diseases. in: Principles of silkworm pathology Seri Scientific publishers, Bangalore, India. pp. 44-98.

Ishikawa, Y. and S. Miyajima (1964) Spread of the infectious flacherie in rearing trays of silkworm, *Bombyx mori* L. *Appl. Entomol. Zool.* **8**, 86-88.

Liu S. X. (1984) Identification on the resistance of silkworm, *Bombyx mori* races to six types of silkworm diseases. *Sericologia* **24**, 377-382.

Nataraju, B. (1995) Studies on Diagnosis and prevention of nuclear polyhedrosis in silkworm, *Bombyx mori* L. Ph.D. Thesis. University of Mysore, Mysore, pp. 96.

Prasad. N. R. (1999) Silkworm disease management and its limitations. *Indian Silk* **37**, 7-9.

Samson, M. V. (1987) Bacterial diseases of Silkworm and their control. Ph.D. Thesis, University of Mysore, Mysore, India.

Samson, M. V., M. Baig, S. D. Sharma, M. Balavenkatasubbaiah, T. O. Shashidharan and M. S. Jolly (1990) Survey of the relative incidence of silkworm diseases in Karnataka. *Indian J. Seric.* **29**, 248-254.

Savanurmath, C. J., S.Basavarajappa, S. B. Hinchigeri, S. Ingalgalli, K. K. Singh, K. and R. D. Shankar (1995) Predisposing factors of viral diseases of silkworm, *Bombyx mori* under agro climatic conditions of north Karnataka. *Indian J. Seric.* **34**, 93-99.

Sivaprakasam, N. and Rabindra (1995) Incidence of grasserie in Silkworm, *Bombyx mori* L. in Selected districts of Tamil Nadu. *Indian J. Seric.* **34**, 100-104.

Tanada, Y. (1967) Effect of high temperature on the resistance of insects to infectious diseases. *J. Seric. Sci. Jpn.* **36**, 333-339.