INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

SCREENING OF VARIOUS BIVOLTINE HYBRIDS OF SILKWORM *BOMBYX MORI* L. FOR DISEASE SUSCEPTIBILITY DURING MONSOON SEASON IN UTTAR PRADESH

[a]Ravinder Sharma, [a]Ganie, N.A., [a]Zia. H. Rufaie, [a]Khan, I.L. & [b]Jyoti Parkash
[a]Temperate Sericulture Research Institute, Mirgund, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Post Box No: 674, G.P.O Srinagar, 190001, INDIA
[b]Division of Sericulture SKUAST-Jammu.

ABSTRACT
Bombyx mori has long been reared as a beneficial insect in the sericulture industry and as an experimental laboratory insect. In tropical countries like India, grasserie, (BmNPV) spreads by the occultation bodies (OBs) in the blood cell of infected silkworm, usually high temperature and humidity prevalent in tropical regions is conductive to proliferation of polyhedrosis disease. It is known to occur in all larval instars during all seasons causing 20-50% cocoon crop losses in India. In the present study, four promising bivoltine hybrids will be screened for their susceptibility to BmNPV and BmIFV, Muscardine and other diseases and the susceptibility status has been compared across monsoon season of Uttar Pradesh. This will help in selecting most promising race for monsoon season of which will be least susceptibility to different Silkworm diseases *Viz.*, Grasserie, Bacterial flacherie, Muscardine and % other diseases (DNV, CPV, IFV, Sotto disease) and farmer will face less difficulty during silkworm rearing and will have no fear of attack of diseases. It will lead to further adoption and spread of Sericulture in other districts of Uttar Pradesh.

KEY WORDS: Grasserie, Flacherie, Susceptibility, Muscardine and % other diseases (DNV, CPV, IFV, Sotto disease) breeds.

INTRODUCTION

The diseases are one of the biggest constraints in cocoon production. There are many survey reports from different sericultural areas in India, which have revealed that the cocoon crop loss in mainly due to diseases (Samson *et al*., 1990; Savanurmath *et al.,* 1994; Savanurmath *et al.,* 1995; Nataraju *et al*., 1988; Selvakumar *et al*., 2002). Among silkworm diseases, viral diseases, Nuclear Polyhedrosis disease is caused by a *Bombyx mori* Nuclear Polyhedrosis virus (BmNPV) and infectious flacherie disease is caused by *Bombyx mori* Infectious Flacherie Virus (IFV). Nuclear Polyhedrosis is known as Grasserie and in India it is also known by various local names such as "Halu halu" in Karnataka, Pala purugu in Andhra Pradesh and Pal puchi in Tamil Nadu indicating the milky fluid condition of the haemolymph of the diseased larvae. Grasserie diseases accounts for more than 15% loss in yield and 25-58%in total disease incidence. The occurrence of the disease is more in fourth and fifth instar larvae under natural conditions. The infected silkworm appears completely normal and feeds as usual till they are close to death. About five to six days after infection the intersegmental membranes swell and worm appears to be under stress and exhibit restless behavior. At this stage, various tissues like epidermis, tracheal matrix, fat bodies, haemolymph. The onset of death from time of swelling of inter-segments is relatively rapid usually ranging from a few hours to less than a day. The symptoms of infectious Flacherie are similar to bacterial Flacherie such as loss of appetite, transparent cephalothorax, shrinkage of the body, retarded growth, empty foregut. This follows vomiting of gastric juice and diarrhea and the gut is filled with yellowish brown fluid. Although, viral diseases cause major damage in monsoon seasons but bacterial and fungal diseases also causes loss. *Bombyx mori* has long reared as a beneficial insect in the sericulture industry and as an experimental laboratory insect. In tropical countries like India, grasserie, (BmNPV) spreads by the occultation bodies (OBs) in the blood cell of infected silkworm Nataraju *et al.,* 2000. Historical writings have described catastrophic outbreaks of polyhedrosis disease in regions of Asia and Europe where *Bombyx mori* were reared on a large scale (Bird, 1953). Usually high temperature and humidity prevalent in tropical regions is conductive to proliferation of polyhedrosis disease. It is known to occur in all larval instars during all seasons causing 20-50% cocoon crop losses in India (Chitra *et al.,* 1975; Nataraju *et al.,* 1998; Samson *et al.*, 1990). Disease condition in which a state of physiological equilibrium of an organism with its environment becomes unbalanced due to non- infectious of infectious causes (Funada, 1968).These infectious and spread of diseases (Bhattacharaya, J., 1992; Govindan *et al.,* 1986; Kobra *et al.,* 1967; Ishikawa and Miyajima, 1964; Ishihara, 1965 and Beig *et al.,* 1988; Miyajima, 1977, 1978 & 1979). Several workers have however have also reported that higher incidence of disease (Swami and Nagaraj, 1992) however density of the silkworm larvae (Krishnaswami *et al.,* 1973; Jaiswal *et al.,* 2003). The population density of larvae reared in a unit area has got direct effect on the incidence of grasserie caused by the

polyhedrosis virus (NPV) (Samson, 1992 & 1987). It also well- established fact that, the Grasserie is most common disease in the tropics and temperature climate. In India it has been reported to be extent of 32.9-55.3% among the total silkworm diseases. Grasserie generally attacks the advance stage of worms i.e. the fourth moult and account for more than 15% of the loss of cocoon yield (Anon, 1993; Aratake, Y., 1973; Aruga *et. al.*, 1963). Earlier workers have established superiority of bivoltine exotic races rearing performance. No serious attempts have been made to check the disease susceptibility of bivoltine hybrids to nuclear polyhedrosis, which is one of the major causes of cocoon crop failure. In Uttar Pradesh there are variations in climatic starting from extreme hot to normal temperature to extreme cold. These different variations of climatic are categorized as monsoon seasons, autumn seasons, spring seasons and summer seasons. Different seasons exhibit differences in susceptibility to several diseases of silkworm. The marked differences in susceptibility to several diseases of silkworm. The marked differences in susceptibility are also due to different silkworm races. Uttar Pradesh is a state where there are too many variations in climatic as a result of which during silkworm rearing seasons, there occurs attack of diseases leading to crop loss. To avoid loss, in the present study, five promising bivoltine hybrids and one control bivoltine hybrid will be screened for their susceptibility to BmNPV and BmIFV, Muscardine and other diseases and the susceptibility status has been compared across monsoon season in Lucknow District of Uttar Pradesh. This will help in selecting most promising race for monsoon season which will be least susceptibility to different silkworm diseases *viz.*, Grasserie, Bacterial flacherie, Muscardine and % other diseases (DNV, CPV, IFV, Sotto disease) and farmer will face less difficulty during silkworm rearing and will have no fear of attack of diseases. It will lead to further adoption and spread of sericulture in other districts of Uttar Pradesh.

MATERIALS & METHODS

The present study was conducted controlled conditions at Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, as well as in the field under natural climatic conditions. Various hybrids like $CSR_2 \times CSR_4$, PAM 101 × NB4D2, CA2 × NB_4D_2 , $CSR_{18} \times CSR_{19}$ and $SH_6 \times NB_4D_2$ (as Control) were selected for the experiment to check their diseases susceptibility in Lucknow region during monsoon season. The standard rearing method was adopted as recommended by Krishnaswami, 1994; Pilli, *et al.*, (1980 & 1987) Krishnaswami *et al*. (1971 & 1978) were followed. During rearing, other precautionary measures such as use disinfectants, removal of diseased silkworm larvae, general cleanliness and sanitation techniques advocated by Krishnaswami *et al.*, (1973) and Krishnaswami *et al*., (1978) were followed. Three replications of each treatment containing 400 larvae of each selected hybrid were maintained for the study under both conditions. The same number of worms / replication was also maintained in control. Data was collected for Grasserie, Flacherie, Muscardine and other diseases every day till onset of spinning. Average temperature and humidity was recorded in natural conditions. Data was subjected to statistical method for deriving the results. The experiments were conducted during monsoon season for two consecutive years *i.e.* 2004-2005 and 2005-2006 respectively.

RESULTS

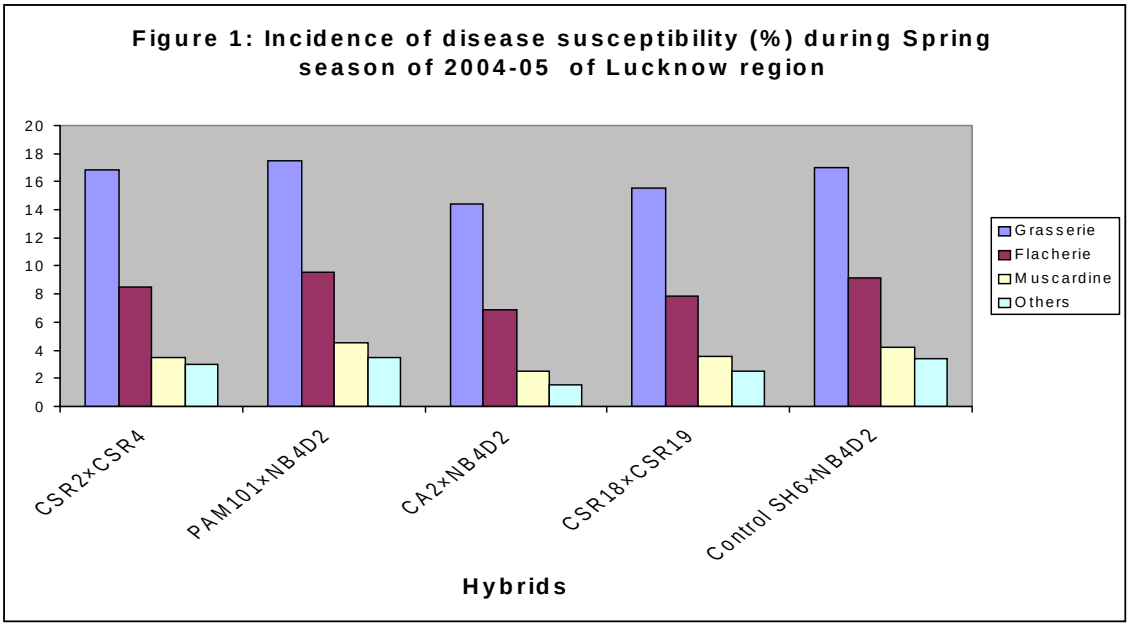
The present study checks the variability in disease susceptibility in four bivoltine silkworm hybrid breeds *i.e.* $CSR_2 \times CSR_4$, $PAM_{101} \times NB4D_2$, $CA_2 \times NB_4D_2$, $CSR_{18} \times CSR_{19}$ and $SH_6 \times NB_4D_2$ (as Control). The observations regarding % of grasserie infected silkworm and % of other diseases (CPV, Densonucleoses viral DNV, infection Flacherie Viral IFV, Sotto Disease) among different bivoltine hybrids in three different replicates in monsoon season of Lucknow are presented in Table below.

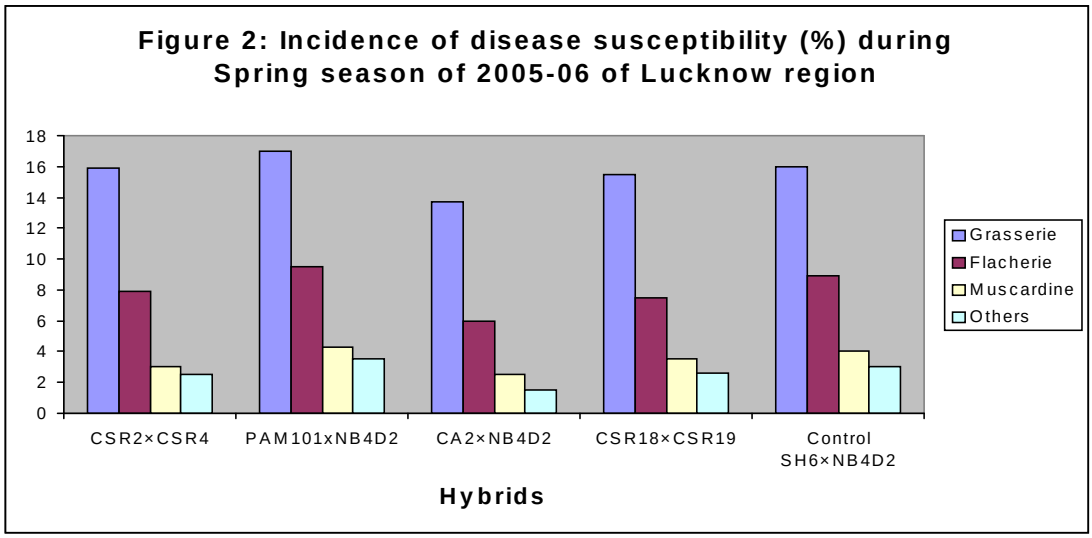
Least % of grasserie was found in $CA_2 \times NB_4D_2$ (14.45); followed by $CSR_{18} \times CSR_{19}$ (15.56); (16.84) in $CSR_2 \times CSR_4$, (17.50) in $PAM_{101} \times NB_4D_2$, and PAM $_{101} \times NB_4D_2$, showed highest grasserie infection than the control race $SH_6 \times NB_4D_2$ (17.00) in the year 2004-05. In year 2005-2006, least % of grasserie infection was found in $CA_2 \times NB_4D_2$ (13.75); followed by (15.50) in $CSR_{18} \times CSR_{19}$, (15.90) in $CSR_2 \times CSR_4$; (17.00) in PAM $_{101} \times NB4D_2$, and $PAM_{101} \times NB4D_2$, showed highest grasserie infection than the control race $SH_6 \times NB_4D_2$ (16.00). Least % of flacherie was found in $CA_2 \times NB_4D_2$ (9.56); followed by (7.82) in $CSR_{18} \times CSR_{19}$; (8.52) in $CSR_2 \times CSR_4$; (9.56) in $PAM_{101} \times NB4D_2$, which depicted highest flacherie infection than the control race $SH_6 \times NB_4D_2$ (9.15) in the year 2004-05. In year 2005-2006, least % of flacherie infection was found in $CA_2 \times NB_4D_2$ (5.96); followed by (7.52) in $CSR_{18} \times CSR_{19}$, (7.90) in $CSR_2 \times CSR_4$; (9.50) in $PAM_{101} \times NB4D_2$, which showed highest flacherie infection and it is more than the control race $SH_6 \times NB_4D_2$ (8.90).

Least % of Muscardine was found in $CA_2 \times NB_4D_2$ (2.52); followed by (3.50) in $CSR_2 \times CSR_4$; (3.56) in $CSR_{18} \times CSR_{19}$; (4.50) in $PAM_{101} \times NB4D_2$, which showed highest Muscardine infection than the control race $SH_6 \times NB_4D_2$ (3.40) in the year 2004-05. In year 2005-2006, least % of Muscardine infection was found in $CA_2 \times NB_4D_2$ (2.50); followed by (3.00) in $CSR_2 \times CSR_4$;(3.50) in $CSR_{18} \times CSR_{19}$, (4.30) in (9.50) in $PAM_{101} \times NB4D_2$, But $PAM_{101} \times NB4D_2$, showed highest Muscardine infection and it is more than the control race $SH_6 \times NB_4D_2$ (8.90). Least % of other diseases was found in $CA_2 \times NB_4D_2$ (1.52); followed by (2.53) in $CSR_{18} \times CSR_{19}$; (3.00) in $CSR_2 \times CSR_4$; (3.50) in $PAM_{101} \times NB_4D_2$, which showed highest infection to other diseases than the control race $SH_6 \times NB_4D_2$ (3.40) in the year 2004-05. In year 2005-2006, least % of other diseases were found in $CA_2 \times NB_4D_2$ (1.50); followed by (2.50) in $CSR_2 \times CSR_4$; (2.60) in $CSR_{18} \times CSR_{19}$, (3.50) in PAM $_{101} \times NB4D_2$, which depicted highest infection to other diseases than the control race $SH_6 \times NB_4D_2$ (3.00).

TABLE 1: Incidence of disease susceptibility percentage during Spring season of 2004-05 and 2005-06 of Lucknow region.

2004-05				
Hybrids	Grasserie	Flacherie	Muscardine	Others
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean± S.E.
$CSR_2 \times CSR_4$	16.84 ± 1.32	8.52±1.10	3.50 ± 0.84	3.00±0.22
$PAM_{101} \times NB_4D_2$	17.50±1.58	9.56 ± 1.52	4.50 ± 0.98	3.50±0.87
$CA_2 \times NB_4D_2$	14.45 ± 1.21	6.85 ± 0.94	2.52 ± 0.23	1.52±0.14
$CSR_{18} \times CSR_{19}$	15.56± 1.28	7.82 ± 1.23	3.56 ± 0.45	2.53±0.52
Control $SH_6 \times NB_4D_2$	17.00 ±1.45	9.15 ± 1.40	4.25 ± 1.21	3.40±0.0.80
P-value	<0.05	<0.05	<0.05	<0.05
2005-06				
Hybrids	Grasserie	Flacherie	Muscardine	Others
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean± S.E.
$CSR_2 \times CSR_4$	15.90 ± 1.28	7.90± 1.05	3.00± 0.80	2.50±0.0.18
$PAM_{101} x NB_4D_2$	17.00± 1.47	9.50± 1.35	4.30± 0.91	3.50±0.81
$CA_2 \times NB_4D_2$	13.75 ±1.18	5.95± 0.90	2.50 ± 0.21	1.50±0.13
$CSR_{18} \times CSR_{19}$	15.50± 1.30	7.52± 1.19	3.50± 0.42	2.60±0.46
Control SH6×NB4D2	16.00±1.40	8.90± 1.35	4.00± 1.18	3.00±0.72
P-value	<0.05	<0.05	<0.05	<0.05





DISCUSSION

The silkworm breeds tested in the present study have shown varied degree of susceptibility among different hybrids in monsoon season of Uttar Pradesh. Higher prevalence was recorded in monsoon season. The difference with respect to disease prevalence as well as the seasons was significant ($p<0.05$). These observations are in conformity with that of previous ones on the prevalence of the disease throughout the year (Satish, 1987; Chisti and Sohaf, 1990; Samson *et al.,* 1990). The comparative susceptibility of diseases in grasserie was seen in different races during monsoon seasons in both years *i.e.,* 2004-05 and 2005-06. The maximum percentage of larval mortality to grasserie was observed in $PAM_{101}\times NB_4D_2$,(17.50 &17.00) and the minimum percentage in $CA_2 \times NB_4D_2$ (14.45 & 13.75) was recorded during the monsoon season. The high prevalence might be due to persistence of the pathogen that is released into the rearing environment. The study also indicates the possible role of temperature in association with humidity on the prevalence of nuclear polyhedrosis. Results from the Table- 1 indicated that the enhanced incidence of the viral diseases was always associated with the increase in temperature. The significant negative correlation in most of the cases between incidence of the diseases and the relative humidity indicates the association between the enhanced incidence of diseases and the decrease in relative humidity. Possibly, the magnitude of incidence of diseases depends on the proportion of the fluctuations in environment. Our observations are in conformity with those of the other authors (Aruga, 1958; Matsubora *et al*., 1984). $PAM_{101} \times NB_4D_2$, (9.56 & 9.50) and the minimum in $CA_2 \times NB_4D_2$ (6.85 & 5.95) was recorded during the monsoon season. This is in accordance with the observations of Aruga (1971) who reported that flacherie disease was very high in monsoon season and late autumn and less in spring.The comparative susceptibility of diseases in flacherie was seen in different races during monsoon seasons in both years *i.e.* 2004-05 and 2005-06. The silkworm breeds tested in the present study have shown a varied degree of susceptibilities to the infection of *Beuveria bassiana, Aspergillus flavus, Bascillus prodiglosus* were also in some extent. $PAM_{101} \times NB_4D_2$, was the most susceptible in present study and less susceptible in present study and less susceptible race was $CA_2\times NB_4D_2$.This result are in confirmation to the earlier work of another scientist *i.e.* Bhagyalakshmi, 1994; Aruga *et al.,* 1963; Chishti, M. Z. and Sohaf, K. A., 1990; Satish, G., 1984; Selvakumar, *et al*., 2002, where they reported that the bivoltine hybrids were most susceptible to disease infection in monsoon seasons. The present study indicated that the $CA_2\times NB_4D_2$ race was less susceptible among other races. Thus this race can be recommended to farmers of Uttar Pradesh for monsoon Season to avoid loss during silkworm rearing due to attack of diseases. Since it is a tested race and in comparison with other races, this race was less susceptible, farmer can easily adopt this race, this race in practice during monsoon season.

REFERENCES

Anon (1993) Silkwom diseases and control report of the sericulture Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang, Jiangus Provience, PRC.

Aratake, Y, (1973) Strain difference of the silkworm, Bombyx mori L. in the resistance to a nuclear polyhedrosis virus. *J. Seric. Sci. Jpn*, **42** (3), 230-238.

Aruga, H. (1958) Mechanism of the virus resistance in the silkworm *Bombyx mori*; VI. On the relation between the rearing season and the cytoplasmic polyhedrosis, *J. Seric. Sci. Jpn.*, **27** (1), 14-17.

Aruga, H. Watanable, H and Nagano, H. (1963) Interference by the heat inactivated virus on the active virus of the cytoplasmic polyhedrosis in the silkworm *Bombyx mori L. J. Seric. Sci. Japan.*, **32,** 51-57.

Aruga, H. (1971) Cytoplasmic polyhedrosis of silkworm-historical, Economical and Epizootiological aspects. In the cytoplasmic polyhedrosis Virus of silkworm.University of Tokyo Press, Tokyo.

Beig, M., Samson, M.V., Sashdharan, T.O., Sharma, S.D., Ballvenkatasubbiah, M. and Jolly, M.S. (1988) study on the spread of Pebrine after introduction of transovarially infected worms in a colony of silkworm, *(Bombyx mori, L.) Sericologia*, **28**: 75-77.

Bhagyalakshmi, T.A. (1994) Host range and strain virulence of Aspergillus tamari Kita and its pathogenicity to different races of *Bombyx mori L*, M. Sc. (Seri.) Thesis, University of Agricultural Sciences, Banglore, India, pp.86.

Bhattacharya, J. (1992) Some biotic and a biotic silkworm factors influencing the outbreak of viral diseases in silkworm *Bombyx mori L. Indian silk*. **45** (1), 45-46.

Bird, ET. (1953) The use of a virus disease in the biological control of the European Pine Sawfly Neodiprion Sertifer (Goeffr). Can. Entomol., **85**, 437-446.

Chisti, M,Z., and Sohaf K.A., (1990). Studies on the polyhedrol diseases of silkworm, *Bombyx mori L*. in Jammu and Kashmir state. *Indian J. Seric.*, **29**. 155-157.

Chitra, C., Karanth, N.G.K., and Vasantharajan, V.N. (1975) Diseases of mulberry silkworm *Bombyx mori L. J. Sci. Indust.* Res., **34**, 386-401.

Funada (1968) Genetic resistance of the silkworm, *Bombyx mori L.* to an infection of a flacherie virus. *J. Sci. Japan* **37**, 281-287.

Govindan, R. (1986) Studies on Kenchu virus diseases of silkworm Bombyx mori L. Ph.D. thesis, VAS, GKVK, Banglore, p.77.

Ishihara, R. (1965) The spread of pebrine with a colony of the silkworm, *Bombyx mori, L. J. Invertebr. Pathol.* **7**, 126-131.

Ishikawa, Y. and Miyajima, S. (1964) Spread of infectious flacherie in rearing tray of Silkworm, *Bombyx mori, Jap. J. Entomolo. Zool.* **8**, 86-88.

Jaiswal, K., Gangwar, S.K. and Dhami, S.S. (2003) Screening of popular bivoltine hybrid of mulberry silkworm (*Bombyx mori Linn.*) in monsoon seasons of Uttar Pradesh. *Recent Reserches in Sericulture Industry.* pp.108-112.

Kobra, R., Aruga, H. and Watanable, H. (1967) Effect of larval growth on the susceptibility of silkworm, *Bombyx mori L*. to a cytoplasmic polyhedrosis virus. *J. Sericult. Sci. Japan* **36**, 165-168.

Krishnaswami S., Tikko B.L (1971). A comparative study of the performance of pure races currently under rearing in Mysore State. *Indian J. Seric.,* **10**: 66-70.

Krishnaswami S. (1973). New technology of silkworm rearing , CSB, Bangalore.

Krishnaswami S. (1978). New Technology of Silkworm rearing. Bulletin No.2, Central Sericultural Reserch and training Institute, Central Silk Board,India.

Krishnaswami, S. (1994) A practical guide to mulberry silk cocoon production in tropics. Srrmula sericulture Consultants, Bangalore, India, pp. 49-50.

Matsubora, F., Wu Y.L., and Ogeshira, H. (1984). Changes in the resistance to the infection with nuclear polyhedrosis virus induced by low and high temperature treatment in the germ free Silkworm *Bombyx mori L. J. Seric. Sc. Japan,* **53**: 548-552.

Miyajima, S. (1977). Disinfection effect of a new chemical on some silkworm pathogens. *Res. Bull. Aichi. Agric. Res Centre.* **8**. 41-45

Miyajima, S. (1978). Effect of high temperature on the incidence of viral flacherie of the silkworm, *Bombyx mori.* Res. Bull.Aichi.Ken. Agri.Res. centr. Series D. *Seric. Jpn.*, **9**, 45-49.

Miyajima, S. (1979). Spread of densonucleosis in rearing seat of silkworm, *Bombyx mori,* res.Bull Aichi. Agric. Res. Center, **11,** 169-172.

Nataraju, B., R.K. Dutta, M. Balavenkatasubbaiah, M.V. Samson, and V. Sivaprasad (1998) Studies on the prevalence of Nuclear Polyhedrosis in sericultural areas of Karnataka, India. *Indian J. Seric.*, **37**: 154-158.

Nataraju, B. Shivaprasad, V. and Datta, R.K. (2000). Studies on development of oral vaccine against NPV in Silkworm *Bombyx mori L. Sericologia.* **40** (3), 42; 427.

Satish, G. and Govindan, R. (1987) Electron Microscopy of nuclear polyhedrosis virus of Silkworm, *Bombyx mori L. Indian J. Seric.*, **26**: 101-103.

Satish, G. (1984) Ecological and pathological studies on the nuclear polyhedrosis diseases of silkworm, *Bombyx mori L. M.Sc* (Agri). Thesis, USA, Banglore. pp.149.

Samson, M.V., Baig, M., Sharma, S.D., Balavenkatasubbaiah, M., Sasidharan, T.O. and Jolly, M.S. (1990). Surver on the relative incidence of silkworm diseases in Karnatka. *Indian J. Sericult.* **29** (2), 248-254.

Savanurmath, C.J., Bavavarajappa, S. B., Hinchigeri, S., Ingathalli, S.S., Singh K.K and Sanakal, R.D.(1994) Relative incidence of the Silkworm viral diseases in agro climatic zones of Northern Karnataka, India. *Bull. Sericult. Res.*, **5**: 51-55.

Selvakumar, T., Nataraju, B., Balavenkatasubbaiah, M., Sivaprasad, V., Baig. M., Virender Kumar Sharma. S.D., Thiagrajan, V. and Datta, R.K. (2002) A report on the prevalence of Silkworm diseases and estimated crop loss. In advanced in India sericulture research (Eds)

Dandin, S.B. and Gupta, proceeding of the National Conference on the strategies for Sericulture Research and Development, CSR&TI, Mysore, Karnataka, India , Nov.16-18. Pp 354-357.

Shivaprakasham, N. and Rabindra, R.J. (1995). Incidence of grasserie in silkworm *Bombyx mori L* in selected districts of Tamilnadu. *Indian J. Seric*, **34** (2), 102-104.

Swamy, M.T and Nagaraj, M. (1992) Survey of disease occurring in mulberry silkworm rearings around Chandragiri. Sericulture Research, CSR&TI, Mysore Dec-10-11.

Venugopala Pillai, S. (1979) Growth studies in Silkworm *Bombyx mori* L. with Special reference to the economic characters. Ph.D.Thesis, University of Kerla.

Venugopala Pillai, S., Krishnaswami, S., Bhat, D.V.,Ranganath, B.K. (1980) Varietal effect on the development and economic characters of Bombyx mori L. Proc. Sericult. Symp. And Seminar, Coimbatore.

Venugopala Pillai, S., Krishnaswami, S. (1987) Adaptability of Silkworm *Bombyx mori L*. to tropical conditions III. Studies on the effect of high temperature during later development stages of Silkworm. *Indian J. Seric.*, **26**: 63-71.