INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH
© 2004-2020 Society For Science and Nature (SFSN). All Rights Reserved.
www.scienceandnature.org

SCREENING OF BIVOLTINE HYBRIDS OF SILKWORM *BOMBYX MORI L*. AGAINST DISEASE SUSCEPTIBILITY DURING SUMMER SEASON IN UTTAR PRADESH

Ravinder Kumar Sharma
College of Temperate Sericulture, Mirgund, SKUAST-Kashmir
Post Box No: 674, G.P.O Srinagar, 190001, INDIA
*Corresponding author email: drravindersharmaskuast@gmail.com

ABSTRACT
In tropical countries like India, Grasserie, Bacterial flacherie, and other diseases (DNV, CPV, IFV, Sotto disease) Spreads by many parasitic microorganism in the blood cells of the silkworm. It is known to occur in all larval instars during all seasons causing 20-50% cocoon crop losses in India. *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 summer season of Uttar Pradesh. This will help in selecting most promising race for summer 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, Environmental conditions.

INTRODUCTION

Mulberry silkworm, Bombyx mori is prone to several infectious diseases caused by various pathogenic micro, organisms like, Muscardine, flacherie, grasserie, and pebrine. The extent of these diseases in silkworms was reported to be 0.48%, 2.30%, 5.00% and 27.88% due to Muscardine, pebrine, nuclear polyhedrosis and cytoplasmic polyhedrosis respectively (Anon, 1992; Givindan, R., 1986; Haidimani, D.K. and Patil, G.M., 2003; Haque, T. and Berman, A.C., 1992; Haque, T. et.al., 1996; Haiso, T.H. and Hsiao, C., 1973). Though the loss of silkworm crop due to infection of muscardine is generally very low, in severe cases, it may be as high as 70.00% (Samson et.al., 1990). Several fungicides are being used to control the muscardine disease. Crowding of silkworm larvae in rearing bed can lead to stress and retarded growth a result of which the larvae become weak and more susceptible to disease are by rearing them using contaminated trays and seat papers (Miiyajima, 1979). The population density of larvae in unit area had a direct effect on the incidence of grasserie disease and its effect on cocoon parameters (Sivprakasam et. al., 1997).

Silkworm reared under natural conditions of temperature and relative humidity show mortality. Other factors, which were responsible for the occurrence of polyhedral diseased, are unhygienic conditions of the rearing room, poor nourishment, overcrowding of the silkworm and poor ventilation of the rearing room. All these factors increase the susceptibility of the silkworm larvae to the infection. No race is immune completely to diseases (Jayaramaiah and Kuberappa, 1986) and different races of silkworm show variation in their susceptibility to different diseases (Liu Shixin, 1964). The silkworm, Bombyx mori, is exposed to a number of disease-causing microorganisms at different levels, causing the development of diseases and mortality. The grasserie caused by the nuclear polyhydrosis virus of Bombyx mori L. (BmNPV) which is the most common and serious disease. Grasserie caused by Bombyx mori nuclear polyhedrosis virus (BmNPV) is one of the most dreaded viral disease fluctuation in temperature and humidity prevalent in tropical regions is conducive to the proliferation of this disease (Subha Raoet.al., 1991). The infective virus particles are known as virions, which are rod shaped with dimensions of 20x70 to 70x200 millimicrons. (Bergold,1947; 1963; Harrap and Longworth, 1974; Kobayashi and Nakagaki, 1984; Hayashiya, K. at.al., 1969; Sen, R. et.al., 1997, 1999a&b). Generally, more than two virions have been observed inside each polyhedral body (Bergold, 1963; Kozolov et.al., 1975; Maeda, S., 1989; Watanabe,1945 &86; Zargar and Gaffar Sheikh, 2003).

The disease prevails all though the year and in the tropics it is significantly high. In india it has been reported to the extent of 32.9-55.3% among the total silkworm diseases (Nataraju et.al., 1998). Nuclear polyhydrosis virus (grasserie) accounts 15% loss in cocoon yield (Vaidya, 1960). The best approach to prevent an infectious disease to use less susceptible or resistant breeds of host to the pathogen. Insects have been observed to respond differentially to infection by microbial pathogens (Weiser, 1969; Ravinder et.al., Briese, 1981). Such differences are genetically determined and have been studied extensively in silkworm to develop resistant breeds. Extensive studies

have been conducted in India on the influence of various pre-disposing factors responsible for the occurrence of Nuclear Polyhydrosis. High temperature, humidity, overcrowding and starvation are some of the important inducing factors for Nuclear Polyhedrosis (Samson, at.al.,1987; Sarma et.al., 1994; Samson et.al., 1990 and Savanurmath ey.al., 1992). Earlier reports indicate that no silkworm races were immune to grasserie and no significant variation in susceptibility was seen within the bivoltine and multivoltine hybrids (Baig et.al., 1991; Ginjamangsimte, 1990; Jayaramaiah and Kuberappa, 1978). Different races of silkworm showed varying degrees of susceptibility to BmNPV indicating different levels of resistance, Aruga (1961) and Watanabe (1967). Significant advances in research in tropical sericulture have been made through concerted efforts in India and other tropical sericulture countries over the years. A composite tropical sericulture technology has also been evolved to minimize the loss due to diseases in silkworm crops. In view of the inadequate disinfection and maintenance of unhygienic conditions in silkworm rearing areas, the use of disease tolerant silkworm breeds/ hybrids can be better option (Dandin, 2002).

Taking in view of the above related studies, the present study was undertaken to study the disease susceptibility in popular bivoltine silkworm hybrids in luck now conditions, as no such study has been taken earlier to find a race that has low disease susceptibility in summer season of Uttar Pradesh.

MATERIALS AND METHODS

The present study was conducted under controlled conditions during summer season for two consecutive years at Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, as well as in the field under natural climatic conditions. Various hybrids like CSR2 × CSR4, PAM 101 × NB4D2, CA2 × NB4D2 , CSR18 × CSR19 and SH6 × NB4D2 (as Control) were selected for the experiment to evaluate their diseases susceptibility in Lucknow region during summer season. The standard rearing method was adopted as recommended by (Krishnaswami, 1994; 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 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 everyday till onset of summer. Average temperature and humidity was recorded in natural conditions. Data was subjected to statistical method for deriving the results.

RESULTS AND DISCUSSION

The present study checks the variability in disease susceptibility in four bivoltine silkworm hybrid breeds *i.e.*CSR2 × CSR4, PAM101 × NB4D2, CA2 × NB4D2 , CSR18 × CSR19 and SH6 × NB4D2 (as Control). The observations regarding % of grasserie infected Silkworm % of flacherie infected silkworm % of muscardine 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 summer season of Lucknow are presented in Table below.

Least % of grasserie infection was found in CSR18 × CSR19 (12.65) followed by (14.78) in CSR2 ×CSR4, (15.50) in CA2 ×NB4D2 (17.96) in PAM101 × NB4D2, which shows highest grasserie infection than the control race SH6 ×NB4D2 (17.00). In year 2004-05. In year 2005-2006, grasserie infection was found in CSR18 × CSR19; (12.20) followed by (14.00) CSR2 × CSR4 (15.00) in CA2 ×NB4D2 (16.60) in PAM 101 × NB4D2, showed highest grasserie infection than the control race SH6× NB4D2 (14.50).

Least % of flacherie infection was found in CSR18 × CSR19 (3.50) followed by (4.00) in CSR2 ×CSR4 (4.36) in CA2 ×NB4D2 (5.50) in PAM101 × NB4D2, which shows highest flacherie infection than the control race SH6 ×NB4D2 (5.00). In year 2004-05. In year 2005-2006, Least % of flacherie infection was found in CSR18 × CSR19; (2.00) followed by (3.50) CSR2 × CSR4 (3.10) in CA2 ×NB4D2 (5.00) in PAM 101 × NB4D2, which depict highest flacherie infection than the control race SH6× NB4D2 (4.90).

Muscardine infection was found Nil in all the races i.e. in CSR18 × CSR19, CSR2 ×CSR4, CA2 ×NB4D2, PAM101 × NB4D2 and SH6 ×NB4D2 in both year 2004-05 and 2005-06.

Least % of other infection was found in CSR18 × CSR19 (1.00) followed by (2.50) in CSR2 ×CSR4 (2.00) in CA2 ×NB4D2 (2.50) in PAM101 × NB4D2, which shows highest percentage of other infection than control race SH6 ×NB4D2 (2.00). In the year 2004-05. In year 2005-2006, Least % other infection infection was found in CSR18 × CSR19; (Nil) followed by (1.50) CSR2 × CSR4 (1.80) in CA2 ×NB4D2 (2.40) in PAM 101 × NB_4D_2, which depict highest other infection than the control race SH6× $NB4D_2$ (2.00).

TABLE 1: Incidence of disease susceptibility percentage reported during Summer seasons during the year 2004-05 in Lucknow region

Hybrids	Grasserie	Flacherie	**Muscardine
(2004-05)	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
CSR2×CSR4	14.78 ±1.44	4.00 ±0.89	Nil
PAM101×NB4D2	17±1.78	5.50±1.04	Nil
CA2×NB4D2	15.50±1.52	4.36±0.96	Nil
CSR18×CSR19	12.56±1.32	3.50±0.88	Nil
Control SH6×NB4D2	17.00±1.66	5.00±0.98	Nil

***No incidence of muscardine disease reported in the summer season.

TABLE 2: Incidence of disease susceptibility percentage reported during summer seasons during the year 2005-06 in Lucknow region

Hybrids	Grasserie	Flacherie	**Muscardine
(2005-06)	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
CSR2×CSR4	14.00±1.36	3.50±0.78	Nil
PAM101×NB4D2	16.60±1.65	5.00±0.97	Nil
CA2×NB4D2	15.00±1.41	3.10±0.93	Nil
CSR18×CSR19	12.20±1.29	2.00±0.26	Nil
Control SH6×NB4D2	14.50±1.60	4.90±0.91	Nil

***No incidence of muscardine disease reported in the summer season.

Performance of breeds was studied in summer season under field conditions with regard to grasserie, flacherie, Muscardine and other diseases which are presented in table. In the present study the difference with respect to disease prevalence among the areas as well as the seasons was significant. These observations are in conformity with that of previous ones on the prevalence of the disease throughout the year (Satish, 1987; Chisti and Soahf, 1990; Simte, 1990). The high prevalence might be due to the persistence of the pathogens that are released into the rearing environment.

The comparative susceptibility of diseases in grasserie was studied during summer seasons in both years. The maximum percentage of larval mortality to grasserie was observed in PAM101 × NB4D2 (17.96 &16.60) and minimum in CSR18 x CSR19 (12.56 & 12.20) was recorded during summer season. These findings of the author are similar to those of Satish (1987).

Grasserie i.e. nuclear polyhedrosis disease of silkworm, Bombyx mori is known to occur in all larval instars and more commonly in IV and V instars during all the seasons. It is evident that high temperature and low humidity during summer months were the two possible factors for higher incidence of grasserie reported that fluctuation between day and night temperature and relative humidity prevailing in the rearing house were the important causes for NPV infection in silkworm larvae (Samson et.al., 1975, 1987, 1990 and Savanurmath, et.al., 1992).

The present study also indicates the possible role of temperature in association with humidity on the prevalence of nuclear polyhydrosis. It has been reported that the role of temperature, humidity and other factors act as stressor in silkworm B mori (Bergolds, 1958) and the fall armyworm, S. frugiperda (Fuxa and Geaghan, 1983). The present study in the end concludes that the search for source of resistance for BmNPV in silkworm genotypes should be continuous process so that stocks, which are least susceptible, can be identified and utilized in synthesizing productive silkworm breeds relatively less susceptible to NPV. In summer season, it has been found that CSR18X CSR19 has low susceptibility (12.20). This study confirms the study of Satish (1987,1989), Smite (1990) and CSR&TI Annual Report 1990 who recorded higher prevalence of grasserie in summer season. The vies is also supported by, Watanabe and Shimizu (1981) who reported that the nuclear polyhydrosis has become dominated to occur through several prefectures, especially in summer and fall rearing seasons in Japan% in dry zone of Northern Karnataka.

The comparative susceptibility of diseases in flacherie was also studied during summer seasons both years. The maximum percentage of larval mortality to flacherie was observed in PAM101 x NB4D2 (5050 &5.00) and the minimum in CSR18 x CSR19 (3.50 & 2.00) was recorded during summer season. In the nuclear polyhydrosis induced batches, flacherie was comparatively lower and a greater part of the mortality was due to nuclear polyhydrosis. The results of this investigation also clearly show that bivoltine and its combination with bivoltine races are quite resistant to nuclear polyhydrosis. Our findings are similar to Doreswamy,c.at.al., 2003.

The least susceptibility of diseases was seen in Muscardine infection during summer season in both the years. The larval mortality to the Muscardine infection was recorded Nil during summer season. This result was confirmed by the earlier scientists (Narayanaswamy, T,K, & Govindan, R., 1989; Paillot, A., 1930 and Stenhaus, E,A., 1949) who observed that in low humidity the occurance of Muscardine was not found in any races.

The silkworm breeds tested in the present study have shown a varied degree of susceptibility to the other infections, which was found Nil in year 2006. In comparision to other breeds, the bivoltine hybrid CSR18 X CSR19 was not susceptible to other diseases among other breeds. This result was confirmed by the earlier work of Anonymous, 1975; Aoki, K., 1971; Bhagyalakshmi, T.A., 1986; Davis, O.L., 1967; Glaser, R.W., 1925; Israngkiji, L. & Sinchaisri, N, 1973; Kawakami, K. & Mikuni, T., 1973; Kawakami, K., 1957; Krishnaswami, S., 1979. Thus, this race was less susceptible; farmer can easily adopt this race, during summer season.

REFERENCES:

Anonymous (1975) Textbook of Tropical Sericulture. Japan overseas Co-operative volunteers, Tokyo, Japan, pp. 99-501.

Apki, K. (1971) Silkworm diseases in Thailand. Bull. Thai. Seric. Res. Training institute., 1, 102-108.

Anon (1993) Silkworm diseases and control report of the Sericulture Research Institute, Chines Academy of Agricultural Sciences, Zhenjiang, Jiangsu Province, PRC. Academic Press New York, pp. 381-412.

Baig, M., Sharma, S.D., Balavenkatasubbiah (1991) Relative susceptibility of different races of silkworm, *Bombyx mori L***.** to Nuclear Polyhedrosis under natural and induced conditions. Sericologia, 31(3), 417-420.

Bergold, G.H (1963) Fine stracture of some insect viruses. J. Insect. Pathology. 5.111-128.

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

Doreswamy, C., Govindhan, R. and Devaiah, M, C. (2003) ET50 for mortality due to late larval flacherie of Silkworm Bombyx mori L. National seminar on disease and pest management on sericulture, U.A.S, Banglore. Abstract No. 26.

Furuta, Y, (1994) Susceptibility of Indian races of Silkworm, Bombyx mori L. to the nuclear polyhedrosis virus and densonucleosis viruses. Acta Seric. Entomology, 1-10.

Glaser, R.W. (1925) Acquired immunity in silkworm, J. Immunolog., 10, 651-662.

Govindan, R. (1986) Studies on kenchu virus disease of silkworm *Bombyx mori L*. Ph.D Thesis, VAS, GKVK, Banglore, p.70-77.

Haque, T. and Barman, A.C.(1982) A feasibility study of Bivoltine silkworm rearing in Bangladesh. Bull. Sericult. Res., Vol, 3, 18-20.

Haque, T., S. Islam and A.C. Barman (1996) Development of high yielding multivoltine breeds of silkworm, Bombyx mori L. Suitable for unfavourable seasons of Bangladesh. Bull. Sericult. Res. Vol, 7, 71-76.

Hsiao, T, H. and Hsiao, (1973) Benonyl: A novel drug for controlling a microsproridian disease of the alfa weevil.J.Invertebr.Patho., 22:303-305.

Israngkijl, L. and Sinchaisri, N. (1973) Varietal resistance of silkworm to Aspergillus disease. Bull. Thai. Seric. Res. Trg. Inst., 3, 113-115.

Jayaramaiah, M; Kuberppa, G.C,; Devaiah, M.C. and Kotikal, Y.K. (1986) White Muscardine disease of silkworm and its management. Indian silk, 25(8) 15-16.

Kawakami, K. Mikuni T. (1973) Studies on the causative fungi of Aspergillu's disease of the silkworm larvae. Resistance to mercuric fungicides and susceptibility in each larval stage of the silkworm to the infection of Aspergilllus's isolates collected from co-operative rearing houses of young silkworm larvae. Bull. Seric. Expt. Stn., 26, 58-77.

Kawakami, K. (1975) Susceptibility of several varieties of the silkworm, Bombyx mori L. to Aspergillus's disease and germination of fungus spores in larval haemolymph.J. Seric.Sci.Jpn,44; 39-44.

Kozolov, E.A., Levitina, T.L., Sidouroua, N.M., Radanski, Yu. L. and Sersbrayani, S.B. (1975) Comparative Chemical studies of the polyhedral proteins of the nuclear Polyhedrosis proteins of Bombyx mori L. and Galleria mellonella. J Insect Pathol., 25 (1), 3-72.

Krishnaswami, S. (1990) what ails our bivoltine silk production programme Indian Silk, 29(1), 6-14.

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

Krishnaswami, S (1979) Improved method of rearing young age Silkworms., Bull. No. 3, Central Silk Board, Banglore, pp.24.

Krishnaswami, S (1978) New Technology of silkworm rearing. Bulletin No.2 Central sericultural Research and Training Institute, Central silk Board, India.

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

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

Maeda, S. (1989) Expression of foreign genes in insect using Baculovirus vectors. Annu. Rev. Entomoj. J4: 351-372.

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

Nataraju, B., R.K. Datta, M. Baig, M. Balvavenkatasubbaiah, M.V. Samson, and V. Sivaprasad (1998) Studies on the prevalence of Nuclear Polyhedrosis in Sericultural areas of Karnataka, India. Indian J. Seric., 37(2), 154-158.

Narayanaswamy T.K and Govindhan R. (1989) Comparative susceptibility of some breeds of silkworm, *Bombyx mori L*. to Aspergillus flavus Link sericologia, 29 (1) 33-37.

Satish, G. (1984) Ecological and pathological studies on the nuclear polyhedrosis diseases of silkworm, *Bombyx mori L*. M.Sc (Agri) Thesis, USA., Banglore. P.145-149.

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

Samson, M.V., Baigh, M., Sharma S.D., Balavenkatasubbaiah, M., Sasidharan, T.o. and Jolly M.S (1990) Survey on the relative incidence of silkworm diseases in Karnataka. Indian J Sericulture. 29(2)248-254.

Samson, M.V (1987) Bacterial diseases of Silkworm, *Bombyx mori L.* Ph.D Thesis University of Mysore.

Samson, M.V., Ganesh, N.K and Kannath, V. (1975) Field survey of various silkworm diseases in Channapatna and Kolar of Karnataka state. Annual Report SCR&TI, Mysore, pp. 88-96.

Samson, M.V (1992) Silkworm crop protection. Lead paper, National Conference on Mulberry Subculture Research December 10-11, Central Sericultural Research and Traning Institute, Mysore, India

Samson, M.V., Baig, M. and Jolly M.S. (1987) Reshamkeet Oushad- A prospective bed disinfectant against grasserie and Muscardine, Indian Silk, 25(1), 16-19.

Savanurmath, C,J., Bavavarajappa, S., Hinchigeri, S., Ingathalli, S,S,. Singh K.K and Sanaka, R.D. (1992) Relative incidence of the the silkworm viral diseases in agro climatic zones of northen Karnataka, India, Abstract National Conference on Mulberry sericulture Research, CSR&TI mysore, Dec, 10-12.

Shivaprakasham, N., Jayarani, S and Rabindra, R J. (1997) Influnce of spacing on the incidence of grasserie in Silkworm Bombys mori L. Indian J. Seric., 36(1), 72-74.

Subba Rao, G., Chandra, A.K and Bhattacharya, J. (1991) Incidence of crop loss from adopted rearers level in West Bengal due to silkworm diseases. Indian J. Sericulture., 30(2), 167-169.

Watanabe, H. and Maeda, S. (1981) Genetically determined non- susceptibility of the silkworm, Bombyx mori L. to infection with a densonucleosis virus (Densovirus), J. Invertebrate. Pathology. 38, 370-373.

Watanabe, H.(1986) Resistance of the silkworm, Bombyx mori L. to viral infections. Agric., Ecosyst. Environ., 15, 131-139.

Zargar, M.A and Gaffar Sheikh A (2003) Bioefficacy of some systematic fungicides and anti- coccidal anti- malaria drugs for the management of pebrine disease of silkworm, Bombyx mori L. caused by Nosema bombycis naeg. National seminar on disease and pest management on sericulture. U.S.A. Bangalore. Abstract No. 30.