STUDIES ON PEBRINE DISEASE OF SILKWORM (*Bombyx mori* L.)

NISAR AHMAD GANIE

DIVISION OF SERICULTURE

FACULTY OF POST-GRADUATE STUDIES
SHER-E-KASHMIR
UNIVERSITY OF AGRICULTURAL SCIENCES AND TECHNOLOGY OF KASHMIR

2003

STUDIES ON PEBRINE DISEASE OF SILKWORM (*Bombyx mori* L.)

THESIS

SUBMITTED TO

The Faculty of Postgraduate Studies
Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir

By

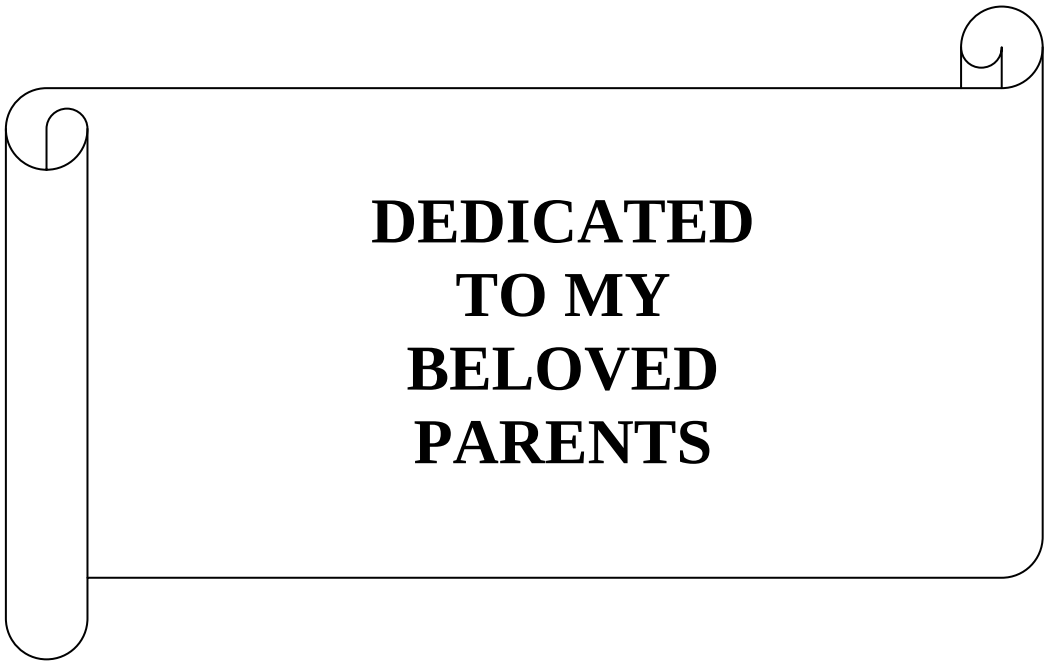
NISAR AHMAD GANIE

In partial fulfilment of the requirements for
award of the Degree of

MASTER OF SCIENCE IN SERICULTURE

Regd. No. 2001-S-7-M

2003



Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Shalimar, Srinagar 191 121

CERTIFICATE-I

This is to certify that the thesis entitled **"Studies on Pebrine Disease of Silkworm, *Bombyx mori* L."** submitted in partial fulfilment of the requirements for the degree of **Master of Science in Sericulture** to the Faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, is a record of *bona fide* research carried out by **Mr. Nisar Ahmad Ganie** (Registration No. 2001-S-7-M) under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of investigation have been duly acknowledged.

Chairman
Advisory Committee

Endorsed:

Professor & Head
Division of Sericulture

SKUAST(K), Mirgund

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Shalimar, Srinagar 191 121

CERTIFICATE-II

We, members of the Advisory Committee of **Mr. Nisar Ahmad Ganie,** candidate for the degree of **Master of Science in Sericulture**, have gone through the manuscript of the thesis entitled **"Studies on Pebrine Disease of Silkworm, *Bombyx mori* L."** and recommend that it may be submitted by the student in partial fulfilment of the requirement for the degree.

Advisory Committee:

Chairman:

Dr. Khursheed Ahmad Sahaf
Assistant Professor
Division of Sericulture
SKUAST (K) Mirgund

Members :

1. **Dr. N. A. Munshi**
   Associate Professor
   Division of Sericulture
   SKUAST (K) Mirgund

2. **Dr. Z. A. Baba**
   Associate Professor

Faculty of Agriculture,
Wadura, Sopore

3. **Dr. M. A. Zargar**
Assistant Professor
Division of Sericulture
SKUAST(K) Mirgund
(Member-cum-Dean PG Nominee)

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Shalimar, Srinagar 191 121

CERTIFICATE-III

This is to certify that the thesis entitled **"Studies on Pebrine Disease of Silkworm, *Bombyx mori* L."** submitted by **Mr. Nisar Ahmad Ganie** (Registration No. 2001-S-7-M) to the Faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, in partial fulfilment of the requirements for the degree of **Master of Science in Sericulture** was examined and approved by the advisory committee and external examiner (s) on 13.3.2004.

External Examiner
Dr. P. N. Chowdhry
Consultant Scientist
National Bureau of Agriculturally Important Micro organisms
Old NBPGR Building IARI,
Complex New Delhi-12

Chairman
Advisory Committee

Professor & Head

Division of Sericulture
SKUAST (K), Mirgund

Director
Resident Instruction-Cum-Dean
Post-Graduate Studies
SKUAST (K) Shalimar

CONTENTS

Chapter	Particulars	Page No.
I.	Introduction	1-4
II.	Review of Literature	5-13
III.	Materials and Methods	14-22
IV.	Experimental Findings	23-55
V.	Discussion	56-61
VI.	Summary and Conclusion	62-63
	Literature Cited	i-vii
	Appendices	

LIST OF TABLES

Table No	Particulars	Page No.
1.	Incidence of Pebrine disease (*Nosema bombycis* N.) of mulberry silkworm (*Bombyx mori* L.) in various Sericultural zones of Kashmir valley (June-July, 2002)	28
2.	Influence of different centrifugation speeds and durations on sedimentation concentration of Pebrine spores (*Nosema bombycis* N.)	34
3.	Improvement in sedimentation concentration of Pebrine spores (*Nosema bombycis* N.) over control	35
4.	Details of chemical evaluated for their influence on fecundity and hatching in *Bombyx mori* L.	38
5.	Influence of some recommended anti-protozoan drugs on fecundity (eggs/female) and hatching percentage of *Bombyx mori* L. (Autumn, 2002)	43
6.	Percent decrease in fecundity (eggs/female) of mulberry silkworm (*Bombyx mori* L.) over check-I (Distilled water)	46
7.	Percent decrease in fecundity (eggs/female) of mulberry silkworm (*Bombyx mori* L.) over check-II (normal/untreated).	49

8. Percent decrease in hatching percentage of mulberry silkworm (*Bombyx mori* L.) over check-I 52

9. Percent decrease in hatching percentage of mulberry silkworm (*Bombyx mori* L.) over check-II 55

LIST OF PLATES

Plate No.	Particulars	After Page No
1	Rearing of silkworms under optimal conditions of temperature and relative humidity i) Rearing of silkworms inside the disinfected rearing room ii) Silkworms in the rearing tray	23
2	Symptoms of Pebrine disease i) Silkworm learvae infected with *Nosema bombycis* ii) Cocoons of Pebrine infected silkworms iii) Silkmoth infected with *Nosema bombycis*	30
3	*Nosema bombycis* spores under microscopic fields i) *Nosema bombycis* spores under 600x magnification ii) *Nosema bombycis* spores under 1500x magnification	31
4.	Staining studies i) Double stained spores of *Nosema bombycis* under 600x microcopic field ii) Double stained spores of *N. bombycis* under 1500x microscopic field.	42
5.	Staining studies i) Safranin stained spores of *N. bombycis* under 600x microscopic field. ii) Safranin stained spores of *N. bombycis* under 1500x	43

microscopic field.

6. Staining studies
   i) Malachite green stained spores of *N. bombycis* under 600x microscopic field
   ii) Malachite green stained spores of *N. bombycis* under 1500 x microscopic field
   iii) 44

7. Staining studies
   i) Methyl blue stained spores on *N. bombycis* under 600x microscopic field
   ii) Methyl blue stained spores *N. bombycis* under 1500x microscopic field 45

8. Staining studies
   i) Crystal Violet stained spores of *N. bombycis* under 600s microscopic field
   ii) Crystal violet stained spores of *N. bombycis* under 1500x microscopic field 46

9. Staining studies
   i) Giemsa stained spores of *N. bombycis* under 600x microscopic field
   ii) Giemsa stained spores of *N. bombycis* under 1500x microscopic field 47

LIST OF APPENDICES

S. No.	Particulars	Appendix No.
1.	Incidence of pebrine disease (*Nosema bombycis* N.) of mulberry silkworm (*Bombyx mori* L.) in various Sericultural Zones of Kashmir valley i) Zone-wise ii) Location-wise	I
	Centrifugation i) Influence of different centrifugation speeds and durations on sedimentation concentration of pebrine spores (*Nosema bombycis* N.) ii) Improvement in sedimentation concentration of pebrine spores (*Nosema bombycis* N.) over control	II
3.	Influence of some recommended antiprotozoan drugs on fecundity (eggs/female) and hatching percentage of *Bombyx mori* L. i) Fecundity ii) Hatching percentage	III

4.	Percent decrease in fecundity i) Over check-I ii) Over check-II	IV
5.	Percent decrease in hatching percentage i) Over check-I ii) Over check-II	V

CERTIFICATE

This is to certify that all the corrections and modifications suggested by the external examiner in the thesis script of **Mr. Nisar Ahmad Ganie** (Registration No. 2001-S-7-M), entitled **"Studies on Pebrine Disease of Silkworm, *Bombyx mori* L."** have been taken care of before final binding of the same.

Dr. Khursheed Ahmad Sahaf
Chairman
Advisory Committee

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir,
Shalimar, Srinagar- 191 121
Division of Sericulture, Mirgund

Tittle of the Thesis : Studies on Pebrine Disease of Silkworm*, Bombyx mori* L.

Name of the Student : Nisar Ahmad Ganie

Admission/Registration No	:	2001-S-7-M
Major Subject	:	Sericulture
Minor Subject (s)	:	Entomology and Plant Pathology
Major Advisor	:	***Dr. Khursheed Ahmad Sahaf***
Degree to be awarded	:	M. Sc. (Sericulture)

ABSTRACT

Pebrine, caused by *Nosema bombycis* N. is a serious disease of mulberry silkworm, *Bombyx mori* L. The disease is an important production constraint of silk in the Kashmir valley. In the present study, conducted in three different Sericultural Zones of Kashmir valley viz., North Zone (Baramulla-Kupawara),Central Zone (Srinagar-Budgam) and South Zone (Anantnag-Pulwama), the disease was found prevalent throughout the Sericultural map of the valley but at varying levels of incidence. The existing rearing practices in the valley were found to have a greater influence on this disease.

For the isolation of pebrine pathogen through the process of centrifugation, maximum sedimentation of spores took place when the centrifugation was carried out at 5000 revolutions for 5 minutes. Among various stains used Safranin, Malachite green and Methyl blue effectively stained the spores whereas Hematoxylin and Eosin gave better visibility when both these stains were applied to the same smear.

All the test chemicals/antiprotozoan drugs evaluated at different concentrations for their influence on fecundity and hatching of *B. mori* were found to record significantly low fecundity (eggs/female) and hatching percentage as compared to check-I and check-II.

ACKNOWLEDGEMENT

"All praises and thanks to "Allah", the Almighty the Most Beneficent , Merciful and Compassionate who gave me courage and strength to go through this crucial juncture".

It gives me immense pleasure to express my deep sense of gratitude to the chairman of my advisory committee, Dr. Khursheed Ahmad Sahaf, Assistant Professor, Division of Sericulture, SKUAST-K, Mirgund under whose kind supervision and encouragement the present research work has been successfully completed. The generous affection, keen scientific interest, constant encouragement, out right help and enthusiastic guidance provided by him has made it possible to accomplish this work.

Words are inadequate to express my deep sense of gratitude, indebtness and heartful thanks to Dr. N. A. Munshi, Associate Professor, Division of Sericulture, SKUAST-K, Mirgund for his whole-hearted support, keen interest, untiring help, generous affection, constant encouragement, wise counselling, meticulous and helpful suggestions during the course of investigations and the preparation of the present manuscript.

My cordial and sincere thanks are due to Dr. M. A. Zargar, Assistant Professor, Division of Sericulture, SKUAST-K, Mirgund for his valuable suggestions, constructive criticism and advice during this study and for finalization of this manuscript.

I express my sincere thanks to my worthy teacher/scientist, Dr. Z. A. Baba, Associate Professor, FOA, Wadura for his valuable comments, all kind of help and moral support during the course of present studies.

I am extremely grateful to Dr. H. U. Dar, Professor and Head, Division of Sericulture, SKUAST-K, Mirgund for his extra ordinary moral encouragement, valuable guidance, constant encouragement which developed in me the spirit of an independent research worker. I also express my venerable gratitude to Dr. Tasneema Kousar, Assistant Professor, Division of Sericulture,

SKUAST-K, Mirgund for her help and valuable suggestions during the course of the study.

The help and cooperation rendered by all the teaching staff members of the Division of Sericulture, Dr. G. N. Malik, Associate Professor, Dr. M. A. Malik, Associate Professor, Dr. A. R. Azad, Assistant Professor, Dr. M. A. Sofi, Associate Professor, Dr. Rayees Ahmad, Assistant Professor, Mr. Aijaz Hamdani, Assistant Professor, Mr. T. P. Singh, Assistant Professor, Mr. Zia-ul-Haq, Assistant Professor, Mr. F. A. Baqal, Assistant Professor, Mr. Sheikh Noor Mohammad, Assistant Professor, Mr. T. A. Raja, Assistant Professor, Mr. F. A. Kakloo, Assistant Professor and Mrs. Sumaira Qayoom, Sr. Technical Assistant and other members is gratefully acknowledged. I am also thankful to Dr. Athar Ali Khan, Assistant Professor, Division of Agri. Stat. and Economics, SKUAST(K), Shalimar for his guidance and assistance in tabulating my data.

The all out help and assistance rendered by the scientific staff and other staff members of Entomology Section of FOA, Wadura is highly commendable. My sincere thanks are due to all scientists and supporting staff of the Division of Plant Pathology, SKUAST(K), Shalimar- particularly to Dr. Shahzad Ahmad, Assistant Professor for their help and co-operation.

I thankfully acknowledge the co-operation and support extended by the Directorate of Sericulture

Development Department, Kashmir and Dr. M. Ashraf Khan, Joint Director, CSR & TI, Pampore.

I extend my heartiest thanks to my friends for their appreciation and nice company and cooperation, Mr. Shafat Ahmad Raja, Ms. Asma Maqbool, Mr. Javid Ahmad Najar, Mr Feroz Ahmad Mattoo, Mr. Ihsan-ul-Haq, Mr. Gulzar Ahmad Ganie, Mr. Ishtiyaq Ahmad Dar, Mr. Mir Rashid Ahmad and Mr. Mir Javaid Iqbal during the period of my study.

I grab for words to express my heartiest gratitude to my esteemed parents for their good wishes, sustained help and encouragement; I extend a note of appreciation to my life partner, Mrs. Nusrat Nisar for her sustained patience, sheer involvement and nice company with out which I probably would not have been able to full-fill such a tough commencement.

Words fail to convey my gratitude to my loving sisters (Saima, Huma and Naseema), Yawar and Osama-Maqbool for their help and blessings.

Lastly, I am thankful to Ms. Saba for showing keen interest in typing and composing this manuscript.

Place: SKUAST(K), Mirgud

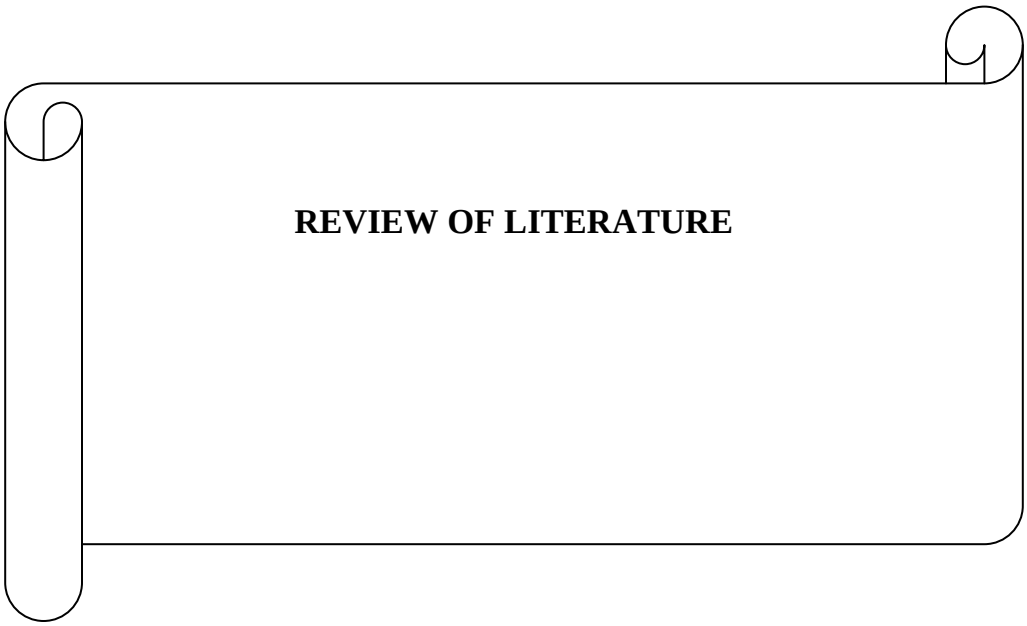
Nisar Ahmad

Dated:

CHAPTER- I

INTRODUCTION

CHAPTER-II



REVIEW OF LITERATURE

CHAPTER- III

MATERIALS AND METHODS

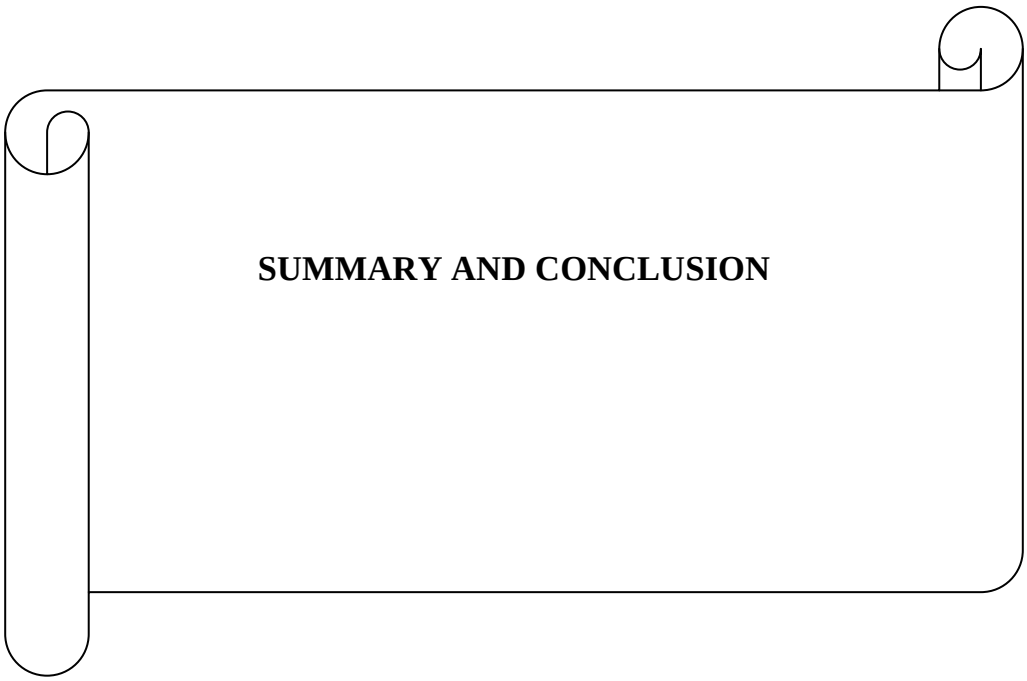
CHAPTER- IV

EXPERIMENTAL RESULTS

CHAPTER- V

DISCUSSION

CHAPTER- VI



SUMMARY AND CONCLUSION

LITERATURE CITED

Chapter-I

INTRODUCTION

Silk is a natural product which has captured the imagination of humanity for over 4000 years. It is an unique combination of fineness, strength, shine and feel. Silk is the queen of fibres, the miracle of thousands of years and old silk. It is interwoven without the traditions and cultures. It enhances the beauty of all that it touches.

Sericulture is an agro-based, labour intensive and export oriented cottage industry. It has been a source of improving socio-economic conditions of the rural populations ever since its discovery. It is a high priority sector for resource allocation in developing countries where relieving unemployment is a prime objective of economic policy. Sericulture has become an inseparable part of Indian culture and tradition. The total raw silk production of the sericulture practising countries of the world has touched 70, 727 metric tonnes in the year 1998 (Anonymous, 2000a) and at present India ranks second in global raw silk production with annual silk production of 15000 MT and is said to increase by 15 per cent per annum to 24000 MT by 2007 (Anonymous, 2003). Although silk production in India has increased enormously, yet the quality of silk does not match the international standards as bulk of the Indian silk comes from multivoltine breeds producing inferior quality silk. The salubrious climatic conditions of Jammu and Kashmir are quite suitable for the production of uni/biovoltine silk of international quality. The Kashmir silk is popular throughout the world because of the ideal agro-climatic conditions for sericulture. But the industry in this state has lost its magnitude due to several

constraints and has been under declining trend during the last few decades with cocoon production reducing from 1.16 million kg/annum in 1955-56 (Anonymous, 1997) to 0.707 million kg/annum in the year 2001-2002 (Anonymous, 2002). The number of families engaged with this job in our state also decreased from 71,700 in 1974-75 to 20,429 in 2001-2002 ((Anonymous, 2002). The total raw silk production in Jammu and Kashmir is reported to be 37000 kg in 2001-2002 (Anonymous, 2002). At present sericulture is practised in 2164 villages of our state (Anonymous, 2002).

The continuous domestication of mulberry silkworm *Bombyx mori* L. since thousands of years has made this insect highly susceptible to various fungal, bacterial, viral and protozoan diseases. These diseases have always proved to be the biggest constraint and hurdle in the progress of sericulture and are responsible for 30-40 per cent crop losses (Kamili and Masoodi, 2000) which nearly neutralizes improvement achieved in the production by the introduction of new technologies. The protozoan disease "Pebrine" caused by *Nosema bombycis* (Naegeli, 1857) is a dreadliest melady in all the sericultural countries of the world. The popular name "Pebrine" was given by Quadrafagues (1860) because of the presence of black pepper like spots on the larval integument. This disease was first recorded in France in 1845 and later spread to other regions of the world (Govindan *et al.*, 1997). The history of sericulture industry reveals the downfall of many sericulturally advanced countries due to the outbreak of this disease. In the state of Jammu and Kashmir pebrine is still an enigma to the progress of sericulture and it was this dreadful disease which almost wiped out the whole silk industry in Kashmir in 1878 when a production indigenous univoltine breed "Kashmir Race" was lost due to the out break of pebrine disease (Lawrence, 1967). The incidence of this disease in Kashmir valley during

1996-1997 has been reported to the extent of 20.0-66.6 per cent (Zargar *et al.,* 2002).

The most frequent source of infection is through ingestion of food (mulberry leaves) contaminated with spores of the parasite, by silkworm larvae or infection from mother moths to its off springs i.e. transovarial transmission (Han and Watanabe, 1988). Also, the disease is to some extent transmitted from one generation to the next through the external contamination of eggs by spores (Transovum transmission). Secondary contamination can also occur through excrement of diseased larvae, contaminated leaf and rearing appliances (Baig *et al.,* 1990; Fujiwara, 1993). In case of transovarial type of infection total crop loss is not uncommon.

The infected worms lose appetite, show irregular moulting and lack behind in growth. Dark brown or black spots appear on the integument once the infection is serious. Pebrinised pupae look flabby and swollen. The infected moths have under develops organs such as legs, wings and distorted antennae, lay more unfertilized eggs in irregular fashion. The eggs laid by the diseased moth lack that adhesive fluid and as such get easily detached from the substrate. Besides, the disease affects moth emergence, reproductive potential and viability (Noamani *et al.,* 1971a; Patil and Geethabai, 1989; Rao, 1998).

Even though the fight against pebrine has continued for more than a century, the loss due to this disease has not been completely eliminated. Keeping in view the importance of this disease it was felt imperative to undertake the study on this disease with the following objectives:

i) To ascertain the incidence of pebrine disease of silkworm *Bombyx mori* L. at cocoon stage in North, South and Central zones of Kashmir valley.

ii) To evaluate suitable techniques for the easy detection of spores of *Nosema bombycis* Naegeli.

iii) To study the influence of recommended antiprotozoan drugs on fecundity and hatching percentage.

Chapter-II

REVIEW OF LITERATURE

The literature pertaining to various aspects of the pebrine disease is briefly reviewed as under:

2.1 Incidence

Noamani *et al.* (1971b) conducted studies on seasonal incidence and intensity of pebrine disease of silkworm under West Bengal climatic conditions for nine different rearing seasons and observed the incidence of the disease in all the seasons. A highest intensity (92.36%) of infection was reported in July-august followed by November-December seasons (84.69%).

Talukdar (1974) studied the incidence of pebrine of Muga silkworm, *Antheraea assamensis* and recorded an average disease incidence of 25.37 per cent.

Chitra *et al.* (1975) carried out their studies in Karnataka to study the seasonal incidence of pebrine. This study lead to the conclusion that pebrine established and spread faster in cool and humid atmosphere than under hot and dry conditions.

Devaiah and Krishnaswamy (1975) reported the appearance of pebrine disease in twenty silkworm races during all the rearing seasons under Mysore climatic conditions. They have reported variation in the percentage of infection in the silkworm races in different seasons. The infection percentage was highest during September-October (38.97%) followed by February-March (32.58%), November-December (14.95%), June-July (6.05%) and the lowest in April-May (4.66%). They have reported a minimum incidence of the disease during the months of April to July when the temperature and humidity were relatively higher and maximum from July –March, when the temperature and humidity were relatively low.

Saha Kundu and Mondal (1975) while carrying out their studies on the incidence of pebrine infection in silkworm eggs from female pebrinised moths at Central Sericulture Research Station, Behrampore reported that the rate of pebrine infection varied from month to month and from season to season. The rate of injection was found to be higher i.e. 86.89 per cent in winter months than in summer months i.e. 72.06 per cent.

Mariswamy and Saikia (1977a) have reported 11.2 per cent of pebrine infection in Muga and Eri silkworms during June-July months in Titabar (Assam). A higher incidence 16.82-18.04 per cent in case of Muga silkworm crops was noticed during April-June in rearing of seed brood than in rearings of commercial crops. However, in case of Eri silkworms the higher incidence of pebrine disease i.e. 8.09 to 86.91 per cent was observed during April-August which decreased gradually in succeeding months till March.

Jolly *et al.* (1981a) have reported 6.95 per cent of pebrine infection from the cocoon samples collected during five months from different cocoon markets located in different regions of Karnataka. Report on the incidence of pebrine in two regions i.e. Attibele and Sarjapura of biovoltine area of Anikal Taluk showed an average percentage of 3.37 and 1.17, respectively in diseased and random samples collected from Attibele area, whereas, in Sarjapura area it was 1.28 and 2.86 per cent in maximum disease incidence i.e. random (3.26%) and diseased samples (7.09%) was reported during September month while minimum i.e. random (0.60%) and diseased samples (1.39%) during the month of October.

Samson *et al.* (1990) conducted survey on the relative incidence of silkworm disease in Karnataka and reported 2-32 per cent of pebrine disease and noted the seasonal variation in the incidence of different diseases. Rainy

and winter seasons were reported the most favourable periods for disease development and prevalence.

Subba Rao *et al.* (1991) reported high incidence of pebrine from May-September and low from October-March in three districts of West Bengal i.e., Birbhum, Mola and Murshidabad during 1987.

Balavenkatasubbaiah *et al.* (1992) reported that in the seed areas of Attibele, Kunigal and Commercial areas Ramnaguram and Chamarajnagar areas, the disease is prevalent in all the areas throughout the year. The infection of the pebrine disease was 25.71 per cent during winter, 5.75 per cent during summer and 15.72 per cent during the rainy season.

Selva Kumar *et al.* (1994) reported 36.34 per cent, 65.54 per cent, 57.18 per cent and 19.88 per cent of pebrine disease in mulberry silkworm during 1990, 1991, 1992 and 1993 respectively, and the prevalence of the disease was high during winter. During 1993-94, pebrine incidence of 7.38 per cent was reported in bivoltine seed cocoons at Central Silk Board, Madiwala (Banglore) which was considered the most alarming situation (Anonymous, 1995).

Zargar *et al.* (2002) carried out his survey studies in the distribution and incidence of pebrine disease in various commercial races/hybrids of silkworm, *Bombyx mori* L. in Kashmir. The highest average incidence of disease (56.6±1.7%) was recorded in Anantnag followed by Baramulla (46.6±2.0%),

Pulwama (44.1±2.5%), Budgam (41.2±2.4%), Kupwara (33.3±2.6%) and Srinagar (25.4±2.1%).

2.2 Techniques for detection of pebrine pathogen

Sato and Watanabe (1980) established the purification method of mature microsporidian spores by iso-density equilibrium centrifugation technique using sucrose or percoll (colloidal silica, pharmacia). After centrifugation of percoll mixed with partially purified *Nosema bombycis* spores in Bechman Type 65 rotor at 73,000g for 30 minutes, a band consisting of mature spores was exclusively obtained. The rate of recovery of mature spores averaged over 90 per cent and no damage to the spores in the course of purification was observed. The also subjected the filtered homogenate to centrifugation and reported that complete sedimentation of spores took place when the centrifugation was carried out at 5000 revolutions for 5-10 minutes.

> The entire silk industry of France would have been wiped out, if not for the timely intervention of Pasteur who gave the concept and method of mother moth examination for the detection of pebrine in 1870 (Anonymous, 1981).

Geetha Bai *et al.* (1985) developed a very simple and inexpensive method at the Karnataka State sericulture Department Institute, by the use of Indian Ink and Potassium Carbonate for an easy detection of pebrine spores and subsequent destruction of infected lots. To a drop of smear placed glaslide a

pin head drop of Indian Ink is added from the edge. After a few minutes the microscopic field becomes black and the spores become shiny thus enabling their easy detection.

Sengupta *et al.* (1993) tried Immuno-Diagnosis of the pebrine disease of silkworm through agar-gel-diffusion, immuno-flourescence and agglutination tests. The agar-gel-diffusion test was negative and no visible precipitation at the zone of equivalence was recorded. A positive result was obtained from the agglutination test which was later confirmed by indirect fluorescent antibody test and ELISA (enzyme-linked immunosorbent assay).

Ananthalakshmi *et al.* (1994b) observed the developmental stages of *N. bombycis* with fresh Giemsa stained smears of the midgut which revealed two and four nucleated schizonts at 48 h pi.

Bansal *et al.* (1997) reported that “Prbrine” infection can be assessed in grainage (egg production units) by examination of testes of male pupae. This method gave more accurate results as the infection was found much higher in testes and ovaries as compared to other tissues

Rekha *et al.* (1998) carried out studies on the group moth inspection of pebrine to understand the effect of different factors such as chemical composition and PH of homogenizing media, duration of homogenization, period of floatation of tissue debris, filtration system and speed and duration of centrifugation 0.5-1.0 per cent potassium carbonate (PH 8-10) and 1.5-2.5 per

cent potassium hydroxide (PH 8-10) were most suitable for homogenizing media. Homogenization for 2 minutes at 9000 rpm was found optimum. Standing of homogenate for 2-3 minutes resulted in good separation of tissue debris from liquid phase. Sedimentation of spores was maximum at 4000-5000 rpm for 3-4 minutes.

Prasad *et al.* (2000) developed a simple, quick method of staining the pebrine spores in the field conditions where a large number of mother moths were examined. When the spores along with the tissue debris were stained using the crystal violet, it took 30 minutes as compared to 60 minutes in case of Giemsa. When the heat fixed smears were pre treated with 20 per cent Potassium permanganate solution, this duration of staining was reduced to one and a half minutes without altering the shape and size of pebrine spores. This method also differentiated the pebrine spores from that of non-pebrine artifacts; the pebrine spores looked deep purple in colour and the rest of the material of homogenate looked in different shades of pink/purple.

2.2 Influence of drugs

Saha Kundu and Mustafi (1980) studied the antimicrosporidial activity of benomyl at central Sericultural Research Station, Behrampur in West Bengal. When the benomyl treated leaves were fed at 4000, 6000 and 8000 ppm concentrations to worms from 3rd instar onwards, reduction in larval mortality due to pebrine infection was observed. Besides, positive effects were observed

as regards the effective rate of rearing, cocoon weight, shell weight and other economic characters.

Jolly *et al.* (1981b) reported that application of antiprotozoan drugs, viz., Fumidil-B and Bavistin at 1500-2000 ppm concentration was effective against the pebrine disease in silkworm.

Chandra and Kundu (1982-83) reported that the growth and multiplication of *Nosema bombycis* N. was kept under check to a great extent when mebandazole an antihelminthic drugs was orally administered at 2000 ppm to B*ombyx mori* larvae. Positive effect of the treatment was observed with respect to larval weight, effective rate of rearing and shell ratio per cent.

Griyaghey *et al.* (1989b) have observed the antipebrine effect of carbestine (0.01-0.1%) and pantelmin (0.1%) and found an increase in effective rate of rearing, cocoon weight, shell weight and shell percentage.

Chandra *et al.* (1995) have reported the efficacy of Bavistin (carbendazim) at 2 to 3 per cent as antimicrosporidial agent in case of *Bombyx mori.* The treatments increased the survival of worms and reduced the pebrine infection. Positive effects have also been reported with respect to larval weight, cocoon weight, cocoon shell weight and shell ratio.

Albendazole at 0.5 per cent concentration has also been reported to reduce the pebrine infection and increase larval weight cocoon shell weight and shell percentage (Anonymous, 1996b).

Bansal *et al.* (1996) studied the effect of various drugs against the microsporidiosis in Tasar silkworm, *Antheraea mylitta* d. and reported that CTR path-12 (unsaturated composition) formulation reduced the larval mortality with a significant improvement in cocoon yield.

Zargar (2001) studied the effect of systemic fungicides and antiprotozoan drugs viz., Bavisting, Topsin-M, Codrinal, Croydoxin-FM, Malariaquine and Metrogyl at various test concentrations against the pebeine disease of silkworm *Bombyx mori* L. and reported that all these chemicals showed a significant control over the disease but did not show any adverse effect upon the health of the silkworm, economic traits of silk and fecundity character. Besides all chemicals were found equally effective at the lowest as well as the highest test concentrations.

Chapter-III

MATERIALS AND METHODS

3.1 Incidence of pebrine disease

The studies with respect to incidence of pebrine disease was carried out in June-July, 2002 in three different Sericultural zones of Kashmir valley viz., North Zone (Baramulla-Kupwara), Central Zone (Srinagar-Budgam) and South Zone (Anantnag-Pulwama). Twenty eight Sericultural villages were randomly selected from all the three zones selecting minimum of five localities from each zone for recording the incidence of pebrine disease. In the North zone, areas surveyed for this purpose include: Botingoo, Malangam, Quilmuqam,

Bonakote, Mundjee, Ayatmulla, Lawaypora, Tapper, Langate, Rawalpora, Nutnosa and Panzgam. Similarly areas surveyed in Central and South Zone include: Newtheed, Kangan, Khimber, Chanapora, Narkura, Modergam, Chattergul, Sarnal, Krad,Brisnoo, Pushnoo, Chedran, Arihal, Rakh, Pirpora and Lassipora. In each village cocoon samples from ten rearers were collected. Simple random sampling procedure was adopted for the collection of samples from the selected areas. From each rearer's house, three samples were taken to represent one treatment. Each sample comprised of ten silkworm cocoons. These samples were preserved in perforated polythene bags separately and kept coded. These samples were later on taken to the laboratory for microscopic examination to confirm the incidence of pebrine disease. The disease incidence was calculate as per the formula adopted by Zargar (2001)

$$\text{Disease incidence (DI)} = \frac{\text{No. of diseased samples}}{\text{Total no. of samples surveyed}} \times 100$$

The data thus generated was subjected to the statistical analysis. During the survey programme, information on the following aspects was also noted:

i) Type rearing

ii) Type of rearing house

iii) Race of the silkworm

iv) Larval spacing

v) Condition of the silkworms

vi) Maintenance of hygrothermic conditions

vii) Application of bed disinfectants

viii) Mode of leaf preservation

ix) Disposal of rearing wastes.

3.2 Techniques for easy detection of Pebrine pathogen

3.2.1 Preparation of the material

Each sample unit (infected pupae) were crushed using pestle and mortar. One to two drops of potassium hydroxide (KOH) solution and one to two drops of 10 per cent potassium carbonate were added to the homogenate, mixed thoroughly and crushed again. Potassium hydroxide softens the tissues by disrupting the cell membranes and potassium carbonate helped in dissolving and minimizing fat globules (Geetha Bai *et al.*, 1985). The spores load (no. of spores/ml) in this mother solution (homogenate) was estimated by using

a haemocytometer (Rekha *et al.*, 1998). In this way a spore suspension of uniform spore load was taken as the parent solution to proceed for further procedures.

3.2.2 Centrifugation

From the mother solution uniform quantity of spore suspension (5 ml) were taken in each replicate (centrifugation tube). For each treatment (rpm) three replications were centrifuged. Centrifugation was carried out at various speeds and for different durations viz.,

Speed:

1000 rpm

2000 rpm

3000 rpm

4000 rpm

5000 rpm

Durations:

1 minute

2 minute

3 minute

4 minute

5 minute

Centrifugation allowed sedimentation of spores present in the homogenate. The sediment was then suspended in uniform quantity (5 ml) of distilled water and the spore number per ml for each replicate was estimated by using haemocytometer. The concentration of spores was calculated as per formula adopted by Dadas *et al.* (1991) for counting the haemocytes.

Total haemocyte count or total spore count =

Haemocytes or pathogen spores in 1 mm square × dilution × depth of the chamber/Number of 1 mm square counted.

3.2.3 Staining method

Different biological stains viz., Giemsa, Delafield Hematoxylin, Eosin, Safranin, Methyl blue, Malachite green, Sudan-III and Crystal violet were used to stain the pebrine spores. Pebrinised moths were used to get spores along with the debris of cell and tissues required for staining. The infected moths were crushed using pestel and mortar. One to two drops of potassium hydroxide solution and one to two drops of 10 per cent potassium carbonate were added to the homogenate mixed thoroughly and crushed again. Potassium hydroxide softens the tissues by disrupting cell membranes and potassium carbonate helped in dissolving and minimizing fat

globules which interfered with visibility of spores (Geetha Bai *et al.*, 1985). Various staining methods both in aqueous and alcoholic solutions were utilized to evolve some suitable techniques for the easy detection of spores of *Nosema bombycis* N. For each stain three replications (slides) were kept in this experimental trial. These slides were coated with a layer of "Mayer's albumin" in order to avoid the splashing of the material during staining. Mayer's albiumin contains egg albumin (50 c.c), glycerol (50 c.c) and thymol (1 g). The coated slides were than allowed to dry for 2-3 minutes. Then a small drop of spore suspension was put on the slide and again allowed to dry for 2-3 minutes. The slides were then passed through different fixatives in order to preserve the morphology of the spore. Fixatives used with their chemical composition include:

Fixative

Chemical composition

i) Methyl alcohol

ii) Cornoy's fixative :

Alcohol (95%) = 60 ml

Chloroform = 30 ml

Glacial acetic acid = 10ml

iii) Gilson's fluid :

Distilled water = 88 ml

Alcohol (95%) = 10 ml

Mercuric chloride = 2 ml

Glacial acetic acid = 0.4 ml

Nitric acid = 1.8 ml

Slides were dipped in the fixative for 2-3 minutes and then allowed to dry up. The material on the slides after fixation was stained. The stains used in the present study include: Giemsa, Delafield

Hematoxylin, Eosin, Safranin, Methyl blue, Malachite green, Sudan-III and Crystal violet. A few drops of the required stain were put over the smear for 1 to 2 minutes, the slide was then washed in running tap water and observed under the microscope. For permanent slides, DPX mountant was used

3.2.3.1 Double staining

The stains used in this process include Delafield, Hematoxylin and Eosin (alcoholic). After fixing, spores were stained with Hematoxylin for 3 minutes and rinsed in water, slide was than passes through 50 per cent and 70 per cent alcohol for 3 minutes each for dehydration and then counter stained with eosin for 3 minutes and washed with 70 per cent alcohol. The slide was next passed through 90 per cent and then through absolute alcohol for 5 minutes in each. For

clearing through two changes of xylene for 5 minutes each and the material was then mounted in Candada balsam.

3.3 Influence of recommended antiprotozoan drugs on the fecundity and hatching percentage of mulberry silkworm, *Bombyx mori* L.

The study was carried out in Autumn season during September, 2002 at Division of Sericulture, Mirgund SKUAST(K). Six important chemicals (Table 4) including a systemic fungicide i.e., [carbendazim (methyl benzimidazole-zylcarbonate Bavistin)] were evaluated for their effect on fecundity and hatching percentage of mulberry silkworm *Bombyx mori* L. These chemicals include:

i) Codrinal :

Sodium salt of P. toluene (β-methoxy ethyl

urethane and tetracycline hydrochloride)

ii) Chloroquine : Chloroquine Phsophate

iii) Mebex : Mebandazole

iv) Orni : Ornidazole

v) Satrogyl : Satranidazole

vi) Bavistin : Carbendazim (methyl benzimidazole-2yl carbamate)

The experiment was laid out in a completely Randomized Block Design (CRBD) with twenty treatments including two checks. Check-I comprised of leaves treated with distilled water and check-II comprised of normal untreated leaves.

For each treatment three replications were kept and each replications comprised of 150 worms of uniform size and age (III-instar).

3.3.1 Preparation of Experiment material

Before starting the experiment, the rearing room and the rearing appliances were disinfected thoroughly with 4 per cent formaldehyde solution. Disease free layings of silkworm hybrid ($NB_4D_2 \times SH_6$) were brushed and the rearing was conducted scientifically as per Dar and Singh, 1998. After III-moult, larvae were counted and distributed among replicates, each replicate comprised of 150 worms of uniform size.

Each chemical was evaluated at three concentrations for their influence on fecundity and hatching percentage. Distilled water was used as a diluant

in every chemical preparation. The antiprotozan drugs were tried at three concentrations viz., 0.1 per cent, 0.2 per cent and 0.4 per cent. The systemic fungicide was tried at 0.25 per cent, 0.5 per cent and 1.0 per cent concentration. The round bottom glass flasks containing these chemicals were coded and plugged with caps. All these operations were performed in a thoroughly disinfected room and later on applied to the mulberry leaf material aseptically.

3.3.2 Application of treatments

The clean and dried mulberry leaves were spread in a thin layer on polythene sheets for feeding to the worms of 18 treatments. Distilled water treated leaves were fed to larval batches of 19th treatment and the normal/untreated leaves were fed to larval batches of 20th treatment.

Proper distance was kept between these 18 leaf feeds to prevent drifting and mixing of chemicals. The corresponding treatment number was labelled to each leaf batch. The various concentrations were thoroughly applied to corresponding leaf batch making sure that no single leaf remains untreated. Hand sprayers were used for applying chemicals. During spraying utmost care was taken to avoid the chemical drift and mixing. For this purpose leaf batches were covered with waste papers which were removed after the spraying was complete to ensure gentle drying of wet leaves.

3.3.3 Feeding of treated leaves to replicated worms

The replicates were labelled with their respective treatment and concentration codes. The treated

leaves were fed to the worms as per their respective treatment and concentration codes. The treated leaves were fed to the replicated larval batches twice during the IV –instar and three times during the V-instar on alternate days, other feeds were of normal untreated leaves.

Distilled water treated leaves were provided to check-I. As a comparison another batch of silkworm, of the same age and size was maintained and fed with normal/untreated leaves. The entire process of rearing was conducted under the optimum conditions of temperature and relative humidity (Plate 1; Fig I & II).

3.3.4 Parameters recorded

At the end of rearing experiment, the following parameters were recorded:

i) Fecundity

After mating the males with females within the same replicate of each treatment, the egg laying capacity of the female silkmoth was then determined as the number of eggs laid by a mother moth.

ii) Hatching percentage

It was calculated as

$$\frac{\text{No. of eggs hatched}}{\text{Total no. of eggs of the laying}} \times 100$$

Chapter-IV

EXPERIMENTAL RESULTS

The present chapter describes the experimental findings of the studies on pebrine disease with respect to incidence, detection of pebrine and Influence of antiprotozoan drugs on fecundity and hatching percentage of mulberry silkworm, *Bombyx mori* L.

4.1 Incidence

4.1.1 Incidence of pebrine disease

Pebrine disease was prevalent in all the locations surveyed with varying degree of incidence (Table 1). The incidence of the disease ranged between 0.025 to 24.96 per cent. As evident from the table the highest i.e., (18.98%) was recorded in South zone followed by North zone (13.46%) and Central zone recorded (9.00 %). All the three zones were found statistically at par with each other, with respect to the disease incidence.

In the North Zone, the incidence of the disease was recorded in the range of 0.025 to 23.50 per cent in various silkworm hybrids viz., $NB_4D_2 \times SH_6$, $CSR_4 \times CSR_2$, $SH_6 \times NB_4D_2$,

NB_4D_2×KA and NB_{18}×SH_6. Amongst the villages Ayatmulla exhibited the lowest incidence (0.025%) as it was the only locality where the silkworm rearing was conducted on scientific basis. Nutnosa suffered the highest (23.50%). The other localities surveyed in this zone with their respective incidence was recorded as: Botingoo (10.19%), Malangam (8.86%), Quilmuqam (9.48%), Bonakote (17.20%), Mundjee (19.16%), Lawaypora (13.57%), Tapper (14.19%), Langate (21.46%), Rawalpora (13.01%) and Panzgam (10.96%).

Among the villages in the central zone, Kangan showed the highest incidence (12.78%) followed by Newtheed (10.93%), Khimber (10.67%), Narkura (5.53%) and Chanapora (5.13%). The incidence of

the disease was recorded in three silkworm hybrids viz., $CSR_4 \times CSR_2$, $SH_6 \times NB_4D_2$ and $NB_4D_2 \times SH_6$ which were commercially reared in these areas.

In the south zone, the incidence was recorded in NB_4D_2(P1), $SH_6 \times NB_4D_2$ and $NB_4D_2 \times SH_6$ silkworm races which were commercially reared in these villages. Among these, the highest incidence (24.96%) was recorded in Chattergul in the $NB_4D_2 \times SH_6$ silkworm hybrid and the lowest (9.14%) in Lassipora in $SH_6 \times NB_4D_2$. The incidence of pebrine in other villages of this zone is as follows: Modergam (20.58%), Sarnal (21.59%), Krad (19.32%), Brisnoo (21.37%), Pushnoo (20.72%), Chedran (23.72%), Arihal (14.60%), Rakh (16.46%) and Pirpora (16.33%).

It is evident from the table that the highest incidence of pebrine (24.96%) was recorded at Chattergul area of South zone and minimum (0.025%) at Ayatmulla of North Zone. However, the disease incidence of 20.58, 21.59, 19.32, 21.37, 20.72, 23.72, 14.60, 16.46, 16.33, 10.93, 12.78, 10.67, 17.20, 19.16, 13.57, 14.19, 21.46, 13.01, 23.50 and 10.96 per cent recorded at Modergam, Sarnal, Krad, Brisnoo, Pushnoo, Chedran, Ariral, Rakh, Pirpora, Newtheed, Kangan, Khimber, Bonakote, Mundjee, Lawaypora, Tapper, Langate, Rawalpora, Nutnosa and Panzgam areas respectively, did not differ statistically from that recorded at Chattergul. Khimber with 10.67 per cent was found statistically at par with that observed at Botingoo, Malangam, Quilmuqam, Ayatmulla,

Lassipora, Chanapora and Narkura which 10.19, 8.86, 9.48, 0.025, 9.14, 5.13 and 5.53 per cent respectively.

4.1.2 Rearing method adopted by farmers

Survey regarding the incidence of pebrine disease (*Nosema bombycis* N.) of mulberry silkworm, *Bombyx mori* L. was carried out during June-July, 2002 in different Sericultural zones of the Kashmir valley, Viz., North Zone (Baramulla-Kupwara), Central Zone (Srinagar-Budgam) and South Zone (Anantnag-Pulwama) were commercial rearing was practised.

During the course of present study, it was found that only one silkworm rearing is practised in the valley at farmers level in the month of May-June. It was also observed that rearing is mostly conducted in villages in an unhygienic and unmaintained

rearing environment making the silkworm larvae susceptible to a number of diseases. It was found that the rearing was conducted in the existing residential houses which can never provide the optimum rearing conditions. Besides, the reares were found least bothered about disinfecting their rearing rooms and rearing appliances and do not have the arrangements for the same. In these houses, disturbance to the worms either intentionally or unintentionally by opening and closing of doors was a routine practice. It was seen that in villages, these rearing houses were surrounded by dense vegetation thus interrupting the supply of fresh air. Windows were not properly maintained to avoid the entry of direct heat of the sun into the rearing room. Generally, the floor type of rearing

was conducted by the rearers. The rearing space was found poorly ventilated and the temperature and humidity were not maintained at their optimum levels. Earthern pots were used for temperature maintenance which produces enough smoke in the rearing house thus telling upon the health of the worms. It was observed that there is tendency among the farmers to overcrowd the worms, either for want of space, rearing equipment or for the purpose of conserving the mulberry leaf forgetting about the fact that overcrowdedness of silkworm larvae leads to their under-nourishment and uneven development worms besides creating conditions favourable for the growth and multiplication of various disease causing organisms. However at some departmental incubation

centres, shelf type of rearing system was adopted which minimized the chances of disease and other hazards. The mulberry leaf used for the rearing of worms was transported from far off places contaminated with dust and other harmful contaminants during transportation and kept heaped on floor of corridors resulting in loss of moisture and subsequent leaf dry age. The farmers were also found least bothered about bed cleaning. If at all the rearing waste was removed, it was found as such in the open atmosphere in their yards. The piling of rearing wastes in the open atmosphere generates injurious gases and favouring multiplication of pathogenic micro-organisms in the vicinity of the rearing rooms/houses which is a strong source of secondary contamination. Dead pebrinised

larvae were also found lying in vicinity of the rearing space which act as a source of secondary infection. Most of the flimsy cocoons collected during survey proved pebrinised after microscopic examination when sometimes moth emergence was allowed from such cocoons, the emerged moths clearly depicted the pebrine symptoms (Plate 2; Fig III). The material collected during the survey showed the presence of spores of *Nosema bombycis* when observed microscopically (Plate 3; Fig I&II).

Due to the above reasons, the silk industry in our state still continues to be under decline despite the high yielding silkworm varieties.

Table 1: Incidence of Pebrine disease (*Nosema bombycis* N.) of mulberry silkworm (*Bombyx mori* L.) in various Sericultural zones of Kashmir valley (June-July, 2002)

S. No.	Seri. Zone/ Location	Race/ hybrid observed	Average disease incidence percent	S. No.	Seri. Zone/ Location	Race/ hybrid observed	Average disease incidence percent
North Zone				**South zone**			
1.	Botingoo	$SH_6 \times NB_4D_2$	10.19 (14.93)	1.	Modergam	$SH_6 \times NB_4D_2$	20.58 (26.68)
2.	Malangam	i) $CSR_4 \times CSR_2$ ii) $NB_4D_2 \times SH_6$	8.86 (13.90)	2.	Chattergul	$NB_4D_2 \times SH_6$	24.96 (29.78)
3.	Quilmuqam	$NB_4D_2 \times SH_6$	9.48 (11.99)	3.	Sarnal	$SH_6 \times NB_4D_2$	21.59 (27.66)
4.	Bonakote	$NB_4D_2 \times SH_6$	17.20 (21.96)	4.	Krad	i) NB_4D_2(P1) ii) $NB_4D_2 \times SH_6$	19.32 (25.72)
5.	Mundjee	$NB_4D_2 \times SH_6$	19.16 (23.24)	5.	Brisnoo	$NB_4D_2 \times SH_6$	21.37 (24.89)
6.	Ayatmulla	$SH_6 \times NB_4D_2$	0.025 (0.90)	6.	Pushnoo	$SH_6 \times NB_4D_2$	20.72 (26.81)
7.	Lawaypora	$NB_4D_2 \times SH_6$	13.57 (19.46)	7.	Chedran	$SH_6 \times NB_4D_2$	23.72 (28.75)
8.	Tapper	$SH_6 \times NB_4D_2$	14.19 (17.46)	8.	Arihal	$SH_6 \times NB_4D_2$ Mirgund	14.60 (20.32)
9.	Langate	i) $CSR_4 \times CSR_2$ ii) $NB_4D_2 \times SH_6$	21.46 (24.87)	9.	Rakh	$SH_6 \times NB_4D_2$	16.46 (21.52)
10.	Rawalpora	$SH_6 \times NB_4D_2$	13.01 (16.90)	10.	Pirpora	$SH_6 \times NB_4D_2$ Mirgund	16.33 (18.98)
11.	Nutnosa	i) $NB_4D_2 \times$ KA ii) $SH_6 \times NB_4D_2$	23.50 (28.66)	11.	Lassipora	$SH_6 \times NB_4D_2$ Tarhama	9.14 (11.75)
12.	Panzgam	i) $NB_4D_2 \times SH_6$ ii) $ND_{18} \times SH_6$ iii) $NB_4D_2 \times SH_6$	10.96 (15.36)		--	--	--

	--	--	**13.46 (17.46)**		--	--	**18.98 (23.89)**
Central Zone							
1.	Newthead	$CSR_4 \times CSR_2$	10.93 (17.52)				
2.	Kangan	$SH_6 \times NB_4D_2$	12.78 (16.61)				
3.	Khimber	$NB_4D_2 \times SH_6$	10.67 (15.27)				
4.	Chanapora	$SH_6 \times NB_4D_2$	5.13 (8.92)				
5.	Narkura	$NB_4D_2 \times SH_6$	5.53 (9.26)				
			9.00 (13.51				

CD (p = 0.05)
Location = (14.61)
Zone = (NS)
*Figures in parenthesis represent arc sine transformed values.

4.2 Techniques for the detection of pebrine pathogen

4.2.1 Centrifugation

Table 2, indicates that with increase in revolutions and durations, there is increase in the sedimentation of spores. Control (uncentrifuged material) recorded the lowest spore load of 4.11×10^5 spores ml^{-1} which was significantly low from all the treatments when tested statistically.

Among the various treatments combinations, the highest number of spores (13.48×10^5 ml^{-1}) was recorded when the centrifugation was carried out at 5000 revolutions for 5 minutes which was statistically at par

with treatments viz., 5000 rpm for 4 minutes, 5000 for 3 minutes, 5000 rpm for 2 minutes, 5000 rpm for 1 minute, 4000 rpm for 5 minutes, 4000 rpm for 4 minutes, 4000 rpm for 3 minutes , 4000 rpm for 2, minutes which recorded spore load to the extent of 13.45×10^5 , 13.37×10^5 , 13.36×10^5, 13.32×10^5, 13.26×10^5, 12.74×10^5, 12.72×10^5 and 12.62×10^5 spores ml^{-1}. 5000 rpm for 4 minutes, 5000 for 3 minutes, 5000 rpm for 2 minutes, 5000 rpm for 1 minute, 4000 rpm for 5 minutes, though statistically at par with each other but differ significantly from 3000 rpm for 5, 3000 rpm for 4 minutes, 3000 for 3 minutes, 3000 rpm for 2 minutes, 3000 rpm for 1 minute, 2000 rpm for 5, 2000 rpm for 4 minutes, 2000 for 3 minutes, 2000 rpm for 2 minutes, 3000 rpm for 1 minute, 1000 rpm for 5, 1000 rpm for 4 minutes, 1000 for 3 minutes, 1000

rpm for 2 minutes, 1000 rpm for 1 minute, which recorded spore load to the extent of 12.27, 10.70, 10.13, 9.60, 9.01, 8.85, 8.53, 8.48, 8.37, 8.00, 7.89, 7.62, 7.46, 7.09 and 6.56×10^{5} spores ml^{-1}. However, 5000 rpm for 4 minutes also differs significantly from 4000 rpm for 1 minute. The values viz., 4000 rpm for 4, 4000 rpm for 3 minutes, 4000 for 2 minutes, 4000 rpm for 1 minute, differed significantly from 3000 rpm for 4 minutes, 3000 for 3 minutes, 3000 rpm for 2 minutes, 3000 rpm for 1 minute, 2000 rpm for 5, 2000 rpm for 4 minutes, 2000 for 3 minutes, 2000 rpm for 2 minutes, 2000 rpm for 1 minute, 1000 rpm for 5, 1000 rpm for 4 minutes, 1000 for 3 minutes, 1000 rpm for 2 minutes and 1000 rpm for 1 minute, 3000 rpm for 4 minutes differed statistically from 3000 rpm for 2 minutes, 3000 rpm for 1 minute, 2000 rpm for 5 minutes, 2000 rpm for

4 minutes, 2000 for 3 minutes, 2000 rpm for 2 minutes, 2000 rpm for 1 minute, 1000 rpm for 5, 1000 rpm for 4 minutes, 1000 for 3 minutes, 1000 rpm for 2 minutes and 1000 rpm for 1 minute. 3000 rpm for 3 minutes and 3000 rpm for 2 minutes though at par with each other but differed significantly from 2000 rpm for 4 minutes, 2000 for 3 minutes, 2000 rpm for 2 minutes, 2000 rpm for 1 minute, 1000 rpm for 5, 1000 rpm for 4 minutes, 1000 for 3 minutes, 1000 rpm for 2 minutes and 1000 rpm for 1 minute, however, 3000 rpm for 3 minutes also differs significantly from 3000 rpm for 1 minute and 2000 rpm for 5 minutes. Statistically at par 3000 rpm for 1 minute and 2000 rpm for 5 minutes differ significantly from 1000 rpm for 4 minutes, 1000 for 3 minutes, 1000 rpm for 2 minutes and 1000 rpm for 1 minute, however, 3000 rpm for 1 minute also differs from

2000 rpm for minute and 1000 rpm for 5 minutes both of which are at par. Statistically at par 2000 rpm for 4 minutes and 2000 rpm for 3 minutes differed significantly from 1000 rpm for 3 minutes, 1000 rpm for 2 minutes an 1000 rpm for 1 minute which are also at par with each other. 2000 rpm for 2 minutes differed from 1000 rpm for 2 minutes and 1000 rpm for 1 2000 rpm for 1 minute.1000 rpm for 5 minutes and 1000 rpm for 4 minutes though at par with each other but differed significantly from 1000 rpm for 1 minute.

4.2.1.1 Improvement in Sedimentation Concentration of Pebrine Spore (*Nosema bombycis* N.) over Control

Statistical analysis of the data reveals that every revolution improved the spore load significantly. Among the revolutions, the highest percent improvement in spore load (69.29%) over control was recorded when the centrifugation was carried out at a speed of 5000 revolutions. This was followed by 4000, 3000, 2000 and 1000 revolutions which recorded percent improvement over control to the extent of 67.58, 60.01, 51.28 and 43.62 per cent which are all statistically dissimilar. Among the durations the highest

percent improvement in spore load (61.27%) over control was recorded when the centrifugation was carried out for 5 minutes which was significantly different from all other durations viz., 1, 2,3 and 4 minutes which recorded percent improvement to the extent of 55.22, 57.29, 58.48 and 59.52 per cent respectively. Among other durations, statistically at par 4 minutes and 3 minutes differed significantly from 1 minute though 4 minutes also differ statistically from 2 minutes. Three and 2 minutes differ significantly from 1 minute, however they are at par with each other.

Among the interactions it was found that every treatment increased the spore load over control but the maximum percent improvement (69.45%) was recorded when the centrifugation was carried out at 5000 r/ 5 min which was tested statistically at par with the treatments viz., 5000 r/ 4 min, 5000 r/ 3 min, 5000 r/ 2 min, 5000 r/ 1 min, 4000 r/ 5 min, 4000 r/ 4 min, 4000 r/ 3 min, 4000 r/2 min, 4000 r/1 min and 3000 r/5 min. which recorded percent improvement to the extent of 69.37, 69.25, 69.23, 69.15, 68.93, 67.65, 67.35, 67.10, 66.86 and 66.49 per cent respectively. All these treatments though at par with each other but differed significantly from some treatments. These treatments from which they differed with their respective percent improvement in spore load over control include:

3000 r/ 4 min (62.72%), 3000 r/3 min (59.33%), 3000 r/2 min (57.17%), 3000 r/1 min (54.35%), 2000 r/5 min (53.56%), 2000 r/4 min (51.82%), 2000 r/3 min (51.52%), 2000 r/5 min

(50.91%), 2000 r/1 min (48.60%), 1000 r/5 min (47.92%), 1000 r/4 min (46.08%), 1000 r/3 min (44.94%), 1000 r/2 min (42.02%), 1000 r/1 min (37.16%) and 3000 r/ 4 min differed significantly from the treatments viz., 3000 r/3 min, 3000 r/2 min, 3000 r/1 min, 2000 r/5 min, 2000 rpm for 4 min, 2000 rpm for 3 min, 2000 r/2 min, 2000 r/1 min, 1000 r/5 min, 1000 r/4 min, 1000 r/3 min, 1000 r/2 min and 1000 r/1 min. 3000 r/3 min and 3000 r/2 min though at par with each other but differed significantly from 2000 r/5 min, 2000 r/4 min, 2000 r/3 min, 2000 r/2 min, 2000 r/1 min, 1000 r/5 min, 1000 r/4 min, 1000 r/3 min, 1000 r/2 min and 1000 r/1 min, however 3000 r/3 min also differ statistically from 3000 r/1 min. 3000 r/1 min itself differs statistically from 2000 r/2 min and 2000 r/1 min, 1000 r/5 min, 1000 r/4 min, 1000 r/3 min, 1000 r/2 min, 1000 r/1 min. 2000 r/3

min differed significantly from 1000 r/5 min, 1000 r/4 min, 1000 r/3 min, 1000 r/2 min and 1000 r/1 min. 2000 r/1 min was statistically dissimilar from 1000 r/3 min, 1000 r/2 min and 1000 r/1 min. 1000 r/3 min and 1000 r/2 min are at par with each other but differ significantly from 1000 r/1 min when tested statistically.

Table 2: Influence of different centrifugation speeds and durations on sedimentation concentration of Pebrine spores (*Nosema bombycis* N.)

S. No.	Treatment		No. of spores × 10^5 ml^{-1}
	Rev.	Dur. (min)	
A.	**1000**		
I.		1	6.560^{i}
II.		2	7.093^{hi}
III.		3	7.467^{h}
IV.		4	7.627^{h}
V.		5	7.893^{gh}
B.	**2000**		
I.		1	8.000^{g}
II.		2	8.373^{g}
III.		3	8.480^{g}
IV.		4	8.533^{g}
V.		5	8.853^{fg}
C.	**3000**		
I.		1	9.013^{f}
II.		2	9.600^{ef}
III.		3	10.133^{de}
IV.		4	10.707^{d}
V.		5	12.273^{bc}
D.	**4000**		

I.	1	12.427^{b}
II.	2	12.627^{ab}
III.	3	12.727^{a}
IV.	4	12.747^{a}
V.	5	13.267^{a}
E.	**5000**	
I.	1	13.327^{a}
II.	2	13.363^{a}
III.	3	13.370^{a}
IV.	4	13.450^{a}
V.	5	13.487^{a}
Check (uncentrifuged)		**4.11^{j}**

CD (p = 0.05) = 0.967

- Each value is a mean of three replicates.
- Values superscripted with similar letter(s) do not differ statistically.

Table 3: Improvement in sedimentation concentration of Pebrine spores (*Nosema bombycis* N.) over control

Duration / *Revolution*	(%) improvement in spore load over control					
	1	**2**	**3**	**4**	**5**	**Mean**
1000	37.16 *$(37.54)^{k}$	42.02 $(40.41)^{j}$	44.94 $(42.09)^{ij}$	46.08 $(42.75)^{hi}$	47.92 $(43.80)^{h}$	43.62 $(41.32)^{ei}$
2000	48.60 $(44.20)^{gh}$	50.91 $(45.52)^{g}$	51.52 $(45.87)^{fg}$	51.82 $(46.04)^{f}$	53.56 $(47.04)^{f}$	51.28 $(45.73)^{di}$
3000	54.35 $(47.50)^{ef}$	57.17 $(49.12)^{de}$	59.33 $(50.38)^{d}$	62.72 $(52.37)^{c}$	66.49 $(54.63)^{ab}$	60.01 $(50.80)^{ci}$
4000	66.86 $(54.85)^{a}$	67.10 $(55.02)^{a}$	67.35 $(55.18)^{a}$	67.65 $(55.34)^{a}$	68.93 $(56.13)^{a}$	67.58 $(55.30)^{bi}$
5000	69.15 $(56.26)^{a}$	69.23 $(56.31)^{a}$	69.25 $(56.32)^{a}$	69.37 $(56.39)^{a}$	69.45 $(56.45)^{a}$	69.29 $(56.39)^{ai}$

Mean	55.22 $(48.07)^{E}$	57.29 $(49.28)^{CD}$	58.48 $(49.97)^{BC}$	59.52 $(50.57)^{B}$	61.27 $(51.61)^{A}$	

CD (p = 0.05)
Revolutions = (0.845)
Durations = (0.845)
Rev. ×Durations= (1.891)

* Figures in parenthesis indicate arc sine transformed values.
- Values superscripted with similar letter(s) are statistically identical.

4.2.2 Staining

Spores of* N. bombycis *isolated from the pebrine material were stained as per the techniques mentioned in "material and methods". Before staining the spores these were treated with the fixative viz., Methyl alcohol, cornoy's fixative and Gilson's fluid, so that their original shape and size is retained during the process of staining. Among the fixatives used in the study, methyl alcohol showed better results as its application retained the original shape and size of the spores during staining process.

The stains used in this study included Giemsa: Delafield Hematoxylin, Eoxin, Safranin, Methyl blue, Malachite green, Sudan-III and Crystal violet. Among these stains Safranin, Malachite green and Methyl blue effectively stained the spores where as Hematoxylin and Eosin gave better results when both these stains were applied successively to the same smear (Double

staining). Here in this case Haematoxylene stained the Muclei of the spore and Eosin stained the cytoplasm. The double stained spores when observed under 600x appear as redish oval shaped against clear background (Plate 4; Fig I) but when observed under high power i.e. 1500x, nuclei appear as dark blue whereas cytoplasm of the spores appear redish in colour (Plate 4; Fig II). This colour differentiation between nuclei and the cytoplasm make detection of spores more easy. Safranin also gave redish colour to the spores and these were clearly visible against the clear background under 600x (Plate 5 ; Fig I), and 1500x (Plate 5; Fig II) microscopic field, as such can easily be detected.

Malachite green and Methyl blue imparted green and blue colourations respectively to the spores. The stained spores in both these cases were easily observed against the clear surrounding under 600x (Plate 6 & 7; Fi I). When observed under high power (1500x) spores stained with malachite green showed dark green sporoplasm whereas anterior and posterior vacuoles of the spores were slightly light green in colour (Plate 6; Fig II). This colour differentiation between sporoplasm and the vacuoles helps in the identification of the spores more easy. Similarly spores stained with crystal violet when observed under high power (1500x) showed dark violet coloured sporoplasm whereas anterior and posterior vacuoles remain almost unstained (Plate 8 ; Fig II) and can easily be detected, though under 600x spores are not easily detectable as some other tissues also get stained with the stain (Plate 8; Fig II) and only careful observation can detect the spores.

Giemsa stain also makes spores easily detectable under high power (1500X) where cytoplasm and the nuclei can be distinguished in the spore, the nuclei stain dark coloured whereas the cytoplasm gets lightly stained (Plate 9 ; Fig II), but under 600x besides spores, fat bodies and other tissues can be seen stained (Plate 9; Fig I), so under this magnification easy detection of the spore is difficult and only careful observation can detect the spores amidst other tissues.

Sudan-III stain was not found as an effective stain as spores remained unstained even when stained for longer durations.

Table 4 : Details of chemical evaluated for their influence on fecundity and hatching percentage of *Bombyx mori* L.

S. No.	Name of the product used		Active ingredient %/Qty.	Concentration used
	Chemical Name	**Trade Name**		
1.	Sodium salt of P. toluene β-methoxyethyl urethane Tetracycline hydrochloride	Codrinal	2-methoxy urethane= 550 mg Tetracycline Hydrochloride = 50 mg	0.4%, 0.2%, 0.1%
2.	Carbendazim Methyl benzimidazole-2yl Carbamate	Bavistin	50 WP	1.0%, 0.5%, 0.25%
3.	Chloroquine phosphate	Chloro-quine	Chloroquine phosphate = 500mg	0.4%, 0.2%, 0.1%
4.	Mebandazole	Mebex	Mebandazole = 100 mg	0.4%, 0.2%, 0.1%
5.	Ornidazole	Orni	Ornidazole = 500mg	0.4%, 0.2%, 0.1%
6.	Satranidazole	Satrogyl	Satranidazole = 300 mg	0.4%, 0.2%, 0.1%

4.3 Influence of recommended antiprotozoan drugs

Influence of some chemicals and a systematic fungicide viz., sodium salt of *P. toluene* [β-methoxy ethyl urenthane and tetracycline hydrocholoride] (Codrinal), Chloroquine phosphate (chloroquine), Mebandazole (Mebx), Ornidazole (Orni), Satranidazole (Satrogyl), Carbendazim (Bavistin) each at three concentration (Table 4) were evaluated in an experimental trial conducted during Autumn, 2000 at Division of Sericulture, SKUAST(K), Mirgund to study their influence on the fecundity (eggs/female) and hatching percentage of mulberry silkworm, *Bombyx mori* L.

4.3.1 Fecundity

Analysis of variance test revealed significantly difference, among all the chemicals evaluated and the two checks with respect to fecundity character. Although the highest number of eggs (615.70 eggs/female) was recorded in check-II but it differs significantly from those recorded in case of all the following chemical combinations.

Codrinal 0.1 per cent (568.40 eggs/female), Codrinal 0.2 per cent (565.53 eggs/female), Codrinal 0.4 per cent (550.63 eggs/female), chloroquine 0.1 per cent (586.50 eggs/female), Chloroquine 0.2 per cent (576.90 eggs/female), Chloroquine 0.4 per cent (557.97 eggs/female), Mebandazole 0.1 per cent (585.90 eggs/female), Mebandazole 0.2 per cent (571.33 eggs/female), Mebandazole 0.4 per

cent (566.57 eggs/female), Ornidazole 0.1 per cent (563.47 eggs/female), Ornidazole 0.2 per cent (561.43 eggs/female), Ornidazole 0.4 per cent (535.73 eggs/female), Satrogyl 0.1 per cent (589.43 eggs/female), Satrogyl 0.2 per cent (587.17 eggs/female), Satrogyl 0.4 per cent (570.67 eggs/female),), Bavistin 0.25 per cent (587.77 eggs/female), Bavistin 0.5 per cent (561.27 eggs/female), Bavistin 1.0 per cent (556.67 eggs/female).

Check-I (Distilled water) with 607.63 eggs/female fecundity, barring satrogyl 0.1 per cent also differ significantly from the above chemical combinations, however check-II was tested statistically at par with check-I. Satrogyl 0.1 per cent, Bavistin 0.25 percent, Satrogyl 0.2 per cent and Chloroquine 0.1 per cent though statistically at par with each other but differ significantly from Mebandazole0.4 per cent, Codrinal 0.2 per cent, Ornidazole 0.1 per cent, Ornidazole 0.2 per cent, Bavistin 0.5 per cent, Chloroquine 0.4 per cent, Bavistin 1.0 per cent, Codrinal 0.4 per cent and Ornidazole 0.4 per cent. However, satrogyl 0.1 per cent, also differs statistically from Cordrinal 0.1 per cent. Mebandazole 0.1 per cent was tested statistically dissimilar from Codrinalf 0.2 per cent, Ornidazole 0.1 per cent, Ornidazole 0.2 per cent, Bavistin 0.5 per cent, chloroquine 0.4 per cent, Bavistin 1.0 per cent, Codrinal 0.4 per cent and Ornidazole 0.4 per cent. Statistically at par Chloroquine 0.2 per cent, Mebandazole 0.2 per cent and Satrogyl 0.4 per cent differ statistically from Codrinal 0.4 per cent and Ornidazole 0.4 per cent both which are at par

however, Chloroquine 0.2 per cent also differ statistically from Bavistin 1.0 per cent.

The lowest fecundity (535.73 eggs/female) was recorded in case of Ornidazole 0.4 per cent which was statistically similar to that observed in case of Codrinal 0.4 per cent (Table 5).

4.3.2 Hatching percentage

Statistical analysis of the data revealed that all the chemicals significantly decreased the hatching percentage when compared to the Check-II. Check-II though recorded the highest hatching percentage (96.65%) but it was significantly different from all the chemical combinations. These chemicals with their respective concentrations and hatching percentage in such batches are as under:

Codrinal 0.1 per cent (86.76%), Codrinal 0.2 per cent (81.30%), Codrinal 0.4 per cent (81.26%), Chloroquine 0.1 per cent (85.14%), Chloroquine 0.2 per cent (84.21%), Chloroquine 0.4 per cent (79.22%), Mebandazole 0.1 per cent (84.70%), Mebandazole 0.2 per cent (83.77%), Mebandazole 0.4 per cent (82.63%), Ornidazole 0.1 per cent (85.44%), Ornidazole 0.2 per cent (83.01%), Ornidazole 0.4 percent (80.19%), Satrogyl 0.1 per cent (85.37%), Satrogyl 0.2 per cent (85.04%), Satrogyl 0.4 per cent (81.23%), Bavistin 0.25 per cent (87.45%), Bavistin 0.5 per cent (86.55%), Bavistin 1.0 per cent (84.79%).

Check-I recorded hatching percentage to the extent of 90.42 per cent

which was significantly different from Check-II, Chloroquine 0.2 per cent, Ornidazole 0.2 per cent, Mebandazole 0.4 per cent, Codrinal 0.4 per cent, Codrinal 0.2 per cent, Satrogyl 0.4 per cent, Ornidazole 0.4 per cent and Chloroquine 0.4 per cent.

Among the chemicals the highest hatching percentage (87.45%) was recorded in case of Bavistin 0.25 per cent which was statistically identical to Codrinal 0.1 per cent, Codrinal 0.2 per cent, Codrinalf 0.4 per cent, Chloroquine 0.1 per cent, Chloroquine 0.2 per cent, Mebandazole 0.1 per cent, Mebandazole 0.2 per cent, Mebandazole 0.4 percent, Ornidazole 0.1 per cent, Ornidazole 0.2 per cent, Satrogyl 0.1 per cen, Satrogyl 0.2per cent, Satrogyl 0.4 per cent, Bavistin 0.5 per cent and Bavistin 1.0 per cent but different from Ornidazole 0.4 per cent and Chloroquine 0.4 per cent. Statistically at par Codrinal 0.1 per cent and Bavistin 0.5 per cent differ significantly from Chloroquine 0.4 per cent.

The minimum hatching percentage (79.22%) recorded in case of Chloroquine 0.4 per cent which tested statistically at par with Ornidazole 0.1 per cent, Satrogyl 0.1 per cent, Chloroquine 0.1 per cent, Satrogyl 0.2 per cent, Bavistin 1.0 per cent, Mebandazole 0.1 per cent, Mebandazole 0.2 per cent, Mebandazole 0.4 per cent, Chloroquine 0.2 per cent, Ornidazole 0.2 per cent, Codrinal 0.2 per cent, Codrinal 0.4 per cent, Satrogyl 0.4 per cent and ornidazole 0.4 per cent (Table 5).

Table 5: Influence of some recommended anti-protozoan drugs on fecundity (eggs/female) and hatching percentage of *Bombyx mori* L. (Autumn, 2002)

Treatment		Fecundity (eggs/female)	Hatching percentage
Chem.	**Conc.**		
Codrinal			
1.	0.1%	568.40[d]	86.76 (68.71)[b]
2.	0.2%	565.53[d]	81.30 (64.48)[c]

3.	0.4%	550.63[ef]	81.26 (64.50)[c]
Chloroquine			
1.	0.1%	586.50[c]	85.14 (67.35)[b]
2.	0.2%	576.90[c]	84.21 (66.59)[c]
3.	0.4%	557.97[d]	79.22 (62.97)[c]
Mebandazole			
1.	0.1%	585.90[c]	84.70 (67.07)[b]
2.	0.2%	571.33[c]	83.77 (66.68)[bc]
3.	0.4%	566.57[d]	82.63 (65.50)[c]
Ornidazole			
1.	0.1%	563.47[d]	85.44 (67.62)[b]
2.	0.2%	561.43[d]	83.01 (66.24)[c]
3.	0.4%	535.73[f]	80.19 (63.58)[c]
Satrogyl			
1.	0.1%	589.43[bc]	85.37 (67.57)[b]
2.	0.2%	587.17[c]	85.04 (67.29)[b]
3.	0.4%	570.67[cd]	81.23 (64.38)[c]
Bavistin			
1.	0.25%	587.77[c]	87.45 (69.31)[b]
2.	0.5%	561.27[d]	86.55 (68.56)[b]
3.	1.0%	556.67[de]	84.79 (67.22)[b]
Check –I (Distilled water Treated leaves)		607.63[ab]	90.42 (72.07)[b]
Check –II (Normal, untreated feed)		615.70[a]	96.65 (79.81)[a]
CD p<0.05		**19.83**	**5.45**

- Figures in parenthesis represent arc sine transformed values
- Each value is a mean of three replicates.
- Values within columns superscripted with similar letter(s) are statistically are at par with each other (p>0.05).

4.3.3 Percent decrease in fecundity over check-I

It is evident from the Table 6 that most of the chemicals have uniformly declined the fecundity (eggs/female) over check-I when tested statistically. However, the maximum per cent decline (8.84%) was recorded in case of Ornidazole and the lowest (4.08%) in case of Satrogyl. Among the other chemicals Codrinal recorded 7.53 per cent decline, Chloroquine (5.51%), Mebandazole (5.40%) and Bavistin (6.36%) decline in fecundity over check-I.

It is also obvious that decline in fecundity increased significantly with increase in concentration of chemicals. It was maximum (8.38%) at 0.4 per cent and minimum (4.45%) at 0.1 per cent. 0.4 per cent though statistically at par with 0.2 per cent but differ statistically from 0.1 per cent. 0.2 per cent was tested at par with 0.1 per cent.

Most of the treatment combinations were found to behave uniformly as they were found to register similar declining effect when tested statistically. However, among them the maximum percent decline (11.78%) in fecundity over check-I was recorded in case of Ornidazole 0.4 per cent and the minimum (2.93%) in case of Satrogyl 0.1 per cent. The other chemical combination viz., Codrinal 0.1 per cent, Codrinal 0.2 per cent, Codrinal 0.4 per cent, Chloroquine 0.1 per cent, Chloroquine 0.2 per cent, Chloroquine 0.4 per cent, Mebandazole 0.1 per cent, Mebandazole 0.2 per cent, Mebandazole 0.4 per cent, Ornidazole 0.1 per cent, Ornidazole 0.2 per cent, Satrogyl 0.2 per cent, Satrogyl 0.4 per cent, Bavistin 0.25 per cent, Bavistin 0.5 per cent and Bavistin 1.0 per cent recorded per cent decline in fecundity over check-I to the extent of 6.40, 6.86, 9.34, 3.41, 5.01, 8.11, 3.52, 5.95, 6.73, 7.21, 7.55, 3.30, 6.02, 3.22, 7.56 and 8.30 per cent respectively.

Table 6: Percent decrease in fecundity (eggs/female) of mulberry silkworm (*Bombyx mori* L.) over check-I (Distilled water).

Conc.(%) / *Treatment*	(%) decrease			
	0.1	**0.2**	**0.4**	**Mean**

Codrinal	6.40 *(14.39)	6.86 (14.87)	9.34 (17.74)	7.53 (15.67)
Chloroquine	3.41 (9.11)	5.01 (12.78)	8.11 (16.37)	5.51 (12.75)
Mebandazole	3.52 (10.25)	5.95 (14.01)	6.73 (14.90)	5.40 (13.05)
Ornidazole	7.21 (14.82)	7.55 (15.40)	11.78 (20.01)	8.84 (16.74)
Satrogyl	2.93 (8.08)	3.30 (9.38)	6.02 (13.92)	4.08 (10.46)
Bavistin**	3.22 (9.91)	7.56 (15.44)	8.30 (16.23)	6.36 (13.86)
Mean	4.45 $(11.09)^{B}$	6.04 $(13.65)^{AB}$	8.38 $(16.53)^{A}$	

CD (p = 0.05)

Chemical = (NS)
Concentration = (3.02)
Chemical×Concentration = (NS)

* Figures in parenthesis represent arc sine transformed values
- Values superscripted with similar letter(s) are statistically at par with each other.

** Bavistin was used at 0.25, 0.5 and 1.0 per cent concentrations.

4.3.4 Percent decrease in fecundity over check-II

All the chemicals was found to register statistically uniform per cent decrease, in fecundity over check-II. However, the maximum per cent decline (10.03%) was recorded in case

of Ornidazole and the minimum (5.36%) in case of Satrogyl. Among the other chemicals Codrinal recorded 8.75 per cent decrease Chloroquine (6.75%), Mebandazole (6.64%) and Bavistin (7.60%).

Table 7 clearly indicates that per cent decline in fecundity increase with the increase in concentration of chemicals/antiprotozoan drugs. It was maximum (9.60%) at 0.4 per cent and minimum (5.70%) at 0.1 per cent concentration 0.4 per cent was tested statistically dissimilar from 0.2 and 0.1 per cent both of which are statistically at par with each other.

Analysis of variance test revealed non-significant difference among majority of the chemicals evaluated at different concentrations. However, amongst them the maximum per cent decline (12.94%) was recorded in case of Ornidazole 0.4 per cent and the minimum (4.20%) in case of Satrogyl 0.1 per cent. The remaining treatment combinations with their respective dosage and percent decline in fecundity over check –II include:

Codrinal 0.1per cent (7.63%), Codrinal 0.2 per cent (8.09%), Codrinal 0.4 per cent (10.53%), Chloroquine 0.1 per cent (4.68%), Chloroquine 0.2 per cent (6.26%), Chloroquine 0.4 per cent (9.32%), Mebandazole 0.1 percent (4.80%), Mebandazole 0.2 per cent (7.17%), Mebandazole 0.4 per cent (7.95%), Orindazole 0.1 per cent (8.42%), Ornidazole 0.2 per cent (8.75%), Satrogyl 0.2 per cent (4.57%), Satrogyl 0.4 per cent (7.32%), Bavistin 0.25 per cent (4.49%),

Bavistin 0.5 per cent (8.78%) and Bavistin 1.0 per cent (9.52%).

Table 7: Percent decrease in fecundity (eggs/female) of mulberry silkworm (*Bombyx mori* L.) over check-II (normal/untreated)

Conc.(%) / *Treatment*	(%) decrease			
	0.1	**0.2**	**0.4**	**Mean**
Codrinal	7.63 *(15.90)	8.09 (16.35)	10.53 (18.88)	8.75 (17.04)
Chloroquine	4.68 (11.68)	6.26 (14.39)	9.32 (17.65)	6.75 (14.57)
Mebandazole	4.80 (12.45)	7.17 (15.42)	7.95 (16.23)	6.64 (14.70)
Ornidazole	8.42 (16.22)	8.75 (16.72)	12.94 (21.03)	10.03 (17.99)
Satrogyl	4.20 (10.98)	4.57 (11.68)	7.32 (15.56)	5.36 (12.74)
Bavistin**	4.49 (11.93)	8.78 (16.96)	9.52 (17.67)	7.60 (15.52)
Mean	5.70 $(13.19)^{B}$	7.27 $(15.25)^{B}$	9.60 $(17.84)^{A}$	

CD (p = 0.05)

Chemical = (NS)
Concentration = (2.57)
Chemical×Concentration = (NS)

* Figures in parenthesis represent arc sine transformed values
- Values superscripted with similar letter(s) are statistically at par with each other.

** Bavistin was used at 0.25, 0.5 and 1.0 per cent concentrations.

4.3.5 Percent decrease in hatching percentage over check-I

Statistical analysis of the data revealed that all the chemicals have uniformly decreased the hatching percentage, however the highest percentage decrease (8.33%) was recorded in case of Chloroquine and the lowest (4.60%) by Bavistin. Among other chemicals Codrinal recorded 8.04 per cent, Mebandazole (7.40%), Ornidazole (8.31%) and Satrogyl (7.19%).

Table 8 clearly shows that percentage decrease in hatching percentage increased with the increase in the concentration of chemicals/antiprotozoan drugs. Among the concentration the highest per cent decline (9.77%) was recorded at 0.4 per cent concentration which was followed by 0.2 per cent and 0.1 per cent which recorded per cent decrease in hatching percentage to the extent of 7.12 and 5.06 per cent

respectively. 0.4 per cent though at par with 0.2 per cent but differs significantly from 0.1 per cent. Both 0.2 per cent and 0.1 per cent concentration are statistically identical.

Analysis of variance test revealed non-significant difference among majority of the interaction means indicating that various treatment combinations were found to register similar declining effect on this character. Amongst them the maximum per cent decrease (12.36%) was recorded in case of Chloroquine 0.4 per cent and the minimum (3.28%) in case of Bavistin 0.25 per cent. The remaining treatment combinations with their respective concentrations and per cent decrease in hatching percentage over check-I include:

Codrinal 0.1 per cent (3.97%), Codrinal 0.2 per cent (10.12%), Codrinal 0.4 per cent (10.03%), Chloroquine 0.1 per cent (5.82%), Chloroquine0.2 per cent (6.80%), Mebandazole 0.1 per cent (6.26%), Mebandazole 0.2 per cent (7.38%), Mebandazole 0.4 per cent (8.57%), Orindazole 0.1 per cent (5.44%), Ornidazole 0.2 per cent (8.30%), Ornidazole 0.4 per cent (11.20%), Satrogyl 0.1 per cent (5.56%), Satrogyl 0.2 per cent (5.85%), Satrogyl 0.4 per cent (10.15%), Bavistin 0.5 per cent (4.27%) and Bavistin 1.0 per cent (6.26%).

Table 8: Percent decrease in hatching percentage of mulberry silkworm (*Bombyx mori* L.) over -check-I

Conc.(%) / *Treatment*	(%) decrease			
	0.1	**0.2**	**0.4**	**Mean**
Codrinal	3.97 *(10.19)	10.12 (18.43)	10.03 (17.90)	8.04 (15.51)
Chloroquine	5.82 (13.93)	6.80 (14.78)	12.36 (20.27)	8.33 (16.33)
Mebandazole	6.26 (13.33)	7.38 (14.19)	8.57 (16.26)	7.40 (14.59)
Ornidazole	5.44 (13.25)	8.30 (15.02)	11.20 (19.44)	8.31 (15.90)
Satrogyl	5.56 (12.99)	5.85 (12.93)	10.15 (18.43)	7.19 (14.78)
Bavistin**	3.28 (10.24)	4.27 (11.70)	6.26 (14.09)	4.60 (12.01)
Mean	5.06 $(12.32)^{B}$	7.12 $(14.51)^{AB}$	9.77 $(17.73)^{A}$	

CD (p = 0.05)

Chemical	=	(NS)
Concentration	=	(3.68)
Chemical×Concentration	=	(NS)

* Figures in parenthesis represent arc sine transformed values
- Values superscripted with similar letter(s) are statistically at par with each other.

** Bavistin was used at 0.25, 0.5 and 1.0 per cent concentrations.

4.3.6 Percent decrease in hatching percentage over check-II

All the chemicals were found to register similar declining effect when tested statistically, however maximum per cent decline (14.25%) in hatching percentage over check-II was recorded in case of Chloroquine and the minimum (10.67%) in case of Bavistin. Among other chemicals Codrinal recorded 13.98 per cent decline, Mebandzole (13.32%), Ornidazole (14.15%) and Satrogyl (13.18%).

Table 9 clearly indicates that percent decline in hatching percentage

over check-II increased with the increase in concentration of chemicals /antiprotozoan drugs. It was maximum (15.57%) at 0.4 per cent concentration, followed by 0.2 per cent and 0.1 per cent concentrations which recorded per cent decrease in hatching percentage to the extent of 13.02 and 11.18 per cent respectively.

All the treatment combinations were found to register similar declining effect on hatching percentage of *Bombyx mori* L. when tested statistically, however Chloroquine 0.4 per cent registered the highest (18.00%) decline in hatching percentage over check-II and Bavistin 0.25 per cent recorded the lowest (9.45%) decline over check –II. Other chemicals with their respective concentrations and per cent decrease in hatching percentage over check-II include:

Codrinal 0.1percent (10.22%), Codrinal 0.2 per cent (15.79%), Codrinal

0.4 per cent (15.92%), Chloroquine 0.1 per cent (11.90%), Chloroquine 0.2 per cent (12.85%), Mebandazole 0.1 per cent (12.36%), Mebandazole 0.2 per cent (13.19%), Mebandazole 0.4 per cent (14.43%), Orindazole 0.1 per cent (11.52%), Ornidazole 0.2 per cent (13.94%), Ornidazole 0.4 per cent (17.01%), Satrogyl 0.1 per cent (11.65%), Satrogyl 0.2 per cent (12.01%), Satrogyl 0.4 per cent (15.87%), Bavistin 0.5 per cent (10.38%) and Bavistin 1.0 per cent (12.18%).

Table 9: Percent decrease in hatching percentage of mulberry silkworm (*Bombyx mori* L.) over check-II

Conc.(%) / *Treatment*	(%) decrease			
	0.1	**0.2**	**0.4**	**Mean**

Codrinal	10.22 *(18.62)	15.79 (23.11)	15.92 (23.48)	13.98 (21.74)
Chloroquine	11.90 (20.00)	12.85 (21.00)	18.00 (24.97)	14.25 (21.99)
Mebandazole	12.36 (20.48)	13.19 (20.18)	14.43 (22.05)	13.32 (20.90)
Ornidazole	11.52 (19.58)	13.94 (20.16)	17.01 (24.35)	14.15 (21.36)
Satrogyl	11.65 (19.84)	12.01 (20.27)	15.87 (23.28)	13.18 (21.13)
Bavistin**	9.45 (17.54)	10.38 (18.49)	12.18 (19.91)	10.67 (18.65)
Mean	11.18 (19.34)	13.02 (20.53)	15.57 (23.01)	

Chemical = (NS)
Concentration = (NS)
Chemical×Concentration = (NS)

* Figures in parenthesis represent arc sine transformed values
- Values superscripted with similar letter(s) do not differ statistically.

** Bavistin was used at 0.25, 0.5 and 1.0 per cent concentrations.

Chapter-V

DISCUSSION

Pebrine, the deadly disease of silkworm, *Bombyx mori* L. caused by the microsporidian, *Nosema bombycis* N. has greatly influenced the

progress made by the Sericulture industry in the past, resulting in sharp decline in cocoon production. This disease is the biggest constraint to the progress of Sericulture. Hence, in order to increase the production and productivity of mulberry silk, it becomes imperative to possess comprehensive knowledge on various aspects of the disease for its early extermination.

The results of the present investigations on Incidence of the disease, techniques for the detection of pebrine pathogen and influence of antiprotozoan drugs on fecundity and hatching percentage of *B. mori* L. are discussed separately as under:

5.1 Incidence

Observations recorded in the present study revealed that, the pebrine disease was prevalent in all the three Sericultural zones of Kashmir valley during the year 2002 with variable incidence irrespective of the variety/race of the silkworm. The incidence of the disease in the valley during 2002 was recorded in the range of 0.025 to 24.96 per cent (Table 1). Among various zones, the highest incidence (18.98 %) was recorded in South zone followed by North zone and Central zone which recorded disease incidence to the extent of 13.46 and 9.00 per cent respectively. The disease incidence was recorded in case of the following silkworm varieties viz., $NB_4D_2 \times SH_6$, $SH_6 \times NB_4D_2$, $CSR_4 \times CSR_2$, NB_4D_2 (P1), $NB_{18} \times SH_6$ and $NB_4D_2 \times$KA.

In the North zone, the highest incidence of the disease (23.50%) was recorded in Nutnosa and lowest (0.025%) in the Anyatmulla area. In the Central

zone, Kangan with 12.78 per cent incidence suffered the most and Chanapora received the lowest disease incidence of 5.13 per cent. In the

South zone, the maximum incidence (24.96%) was recorded in Chattergul area and the lowest (9.14%) in Lassipora.

Similar studies on the incidence of pebrine disease have been reported from other parts of the country and abroad (Noamani *et al.*, 1971b; Davaiah and Krishnaswamy, 1975; Samson, *et al.*, 1990; Subba Rao *et al.*, 1991; Chitra *et al.*, 1975 and Zargar, 2001). In this study it was found that the infection of the disease was due to secondary contamination as in case of primary infection (transovarian transmission), the larvae do not grow and survive beyond the II-instar (Jolly, 1986; Han and Watanabe, 1988). Besides, during present study, a clear variation in the incidence of the disease was observed between the region and such observations were in agreement to those of Sprague (1977). Among the different zones of the Kashmir valley, Anantnag, Baramulla and Pulwama are the traditional Sericultural areas having more number of villages and silkworm rearers (Anonymous, 1999). Presence of more number of silkworm rearing village/rearers and unscientific method of rearing could be the reasons behind the higher incidence of the disease in such areas.

The inadequacy of rearing houses, lesser rearing space, floor system of rearing houses, unhygienic conditions, lack of disinfectants, improper arrangements for optimum temperature, humidity and ventilation and improper disposal of rearing wastes were the general observations recorded almost every where during the survey study. All these factors make the conditions conducive for the higher incidence of the disease. Utilizing rearing rooms and other rearing appliances without proper disinfection results in the accumulation of disease causing pathogens (Baig *et al.*, 1990). According to Kashkarova and

Khakhanov (1980) *Nosema bombycis* is also found in other lepidopteran insects besides silkworms, so entry of these insects into the rearing rooms should be prevented to avoid infection of silkworm larvae.

The lesser incidence of the disease was found in some departmental rearing centres and in the areas which were directly under the influence of state Sericulture Department as in these areas rearing was based on scientific know how and the rearing was conducted by trained and skilled person under the supervision of concerned department. The shelf type of rearing was practised in most of these areas. Besides, the arrangements for disinfection of rearing rooms and appliances, maintenance of temperature, humidity, ventilation, bed cleaning, spacing, bed disinfection and proper disposal of rearing waste were observed in such areas. These factors were mainly responsible for the low incidence of the disease in such areas.

5.2 Techniques for the detection of pebrine pathogen

Pebrine disease is non-tolerable even at its lowest degrees of incidence. This disease can cause of total crop loss if neglected in the initial stages. So developing an effective methodical kit in order to detect this disease and keep it under check appears to be the need of an hour.

5.2.1 Centrifugation

The purpose of centrifugation is to get maximum number of spores in the sediment from a homogenate which is having low concentration of *Nosema bombycis* spores so that this disease is not overlooked or wrongly diagonsed at any stage. In the present study (13.48×10^5 ml^{-1}) was recorded when a mother solution of 4.11×10^5 spores m^{-1} spore strength was subjected to centrifugation at 5000 revolutions for 5 minutes which was tested statistically at par with 5000 revolution for 4 minutes, 5000

revolution for 3 minutes, 5000 revolution for 2 minutes, 5000 revolution for 1 minute, 4000 revolution for 5 minutes, 4000
revolution for 4 minutes, 4000 revolution for 3 minutes and 4000 revolution for 2 minute and. 4000 revolutions for 1 minute. The results of the present investigations are in confirmity with those obtained by Rekha *et al.* (1998) who observed 4000-5000 revolutions for 3-4 minutes as the most effective and also with those of Sato and Watanabe (1980) who reported 5000 revolutions for 5-10 minutes as effective.

5.2.2 Staining

The purpose of staining was to develop an effective and easy method for the detection of spores of *Nosema bombycis* which otherwise get mingled with the homogenate of the moth like fat bodies, tissue debris etc. especially when the spore load in the worm /moth is very low which leads to wrong diagnosis of disease.

Though there are several other techniques available for the diagnosis of the *Nosema bombycis* like use of antibody sensitized latex (Hayashiya and Ayuzawa, 1987; Watnable, 1987), fluorescent antibody techniques (Sato *et al.,* 1981) and immunodiagnosis (Sengupta *et al.,* 1993). But these methods being complex, expensive, long time procedures and involvement of greater technical skills can not be practised in the field where large number of moths are to be examined within a considerably short period of time. Hence staining methods help in easy detection of spores. In the present study several stains viz., Hematoxylin, Eosin, Safranin, Giemsa, Sudan-III, Malachite green, Methylene blue and Crystal violet were used to screen out the most effective stain. Among these stains

Safranin, Malachite green, Crystal violet and Methylene blue were found effective for easy detection of the spores.

Geetha Bai *et al.* (1985) reported Indian Ink effective but it did not differentiate spores from spore resembling artifacts like fat globules, tissue

debris etc as reported by Prasad *et al.* (2000) who instead found crystal violet effective for the easy detection of spores. Same stains was also observed suitable in the present study. A double staining method comprising Hematoxylin and Eosin can also be helpful in the diagnosis of the disease though it takes comparatively longer time than single staining methods.

5.3 Influence of antiprotozoan and antifungal drugs on fecundity and hatching percentage

Five different antiprotozoan drugs and a systemic fungicide viz., Codrinal, Chloroquine, Mebandozole, Ornidazole, Satrogyl and Bavistin were evaluated each at three different concentrations to study their influence on fecundity and hatching *Bombyx mori.* L.

5.3.1 Fecundity

All the chemicals/antiprotozoan drugs were found to record significantly low fecundity as compared to check-I and check-II. However, the maximum number of eggs/female (589.43) was recorded in case of satrogyl 0.1 per cent and the minimum number of eggs/female (535.73) was recorded in case of Ornidazole 0.4 per cent (Table 5). As per the available literature, the earlier workers (Saha Kundu and Chandra, 1978; Saha Kundu and Mustafi, 1980; Jolly *et al.,* 1981b) have not

mentioned about the effect of various chemicals which they had used for the control of pebrine disease, upon the fecundity character and many other characters like denier, reelibility, bave length etc. Zargar, 2001 studied the antimicrosporidial effect of various chemical viz., Bavistin, Topsin-M, Codrinal, Croydoxin-FM, Malariaquine and Metrogyl and reported that the chemicals showed a significant increase in weight of larval, yield of cocoons and exhibited a positive influence upon all the economic traits and fecundity. Regarding the fecundity character, the present findings are not in conformity with that of Zargar (2001) as the results on this aspect were generated in a single experimental trial. However, in the present study, the effect of these chemicals on fecundity character was found significant as compared to the check-I and check-II.

5.3.2 Hatching percentage

The chemicals evaluated for their influence on the hatching percentage of mulberry silkworm, *Bombyx mori* L. showed significant effect upon this character. It was found that there is significant variation in the hatching percentage recorded in all the treated batches and the two checks. However, the maximum hatching percentage (87.45%) was recorded in case of Bavistin 0.25 per cent and the minimum (79.22%) was recorded in case of Chloroquine 0.4 per cent.

Jolly *et al.*, 1981b; Chandra and Kundu, 1982-83; Liu Shi Xian, 1987; Griyaghey *et al.*, 1987 have reported the efficacy of Carbendazim and other chemicals against the pebrine infection in silkworm. However, they have not studied these chemicals in relation with characters like fecundity and hatching percentage. In the present study, all the chemicals showed significant effect upon these characters.

Chapter-VI

SUMMARY AND CONCLUSION

The investigations on the incidence of pebrine, techniques for the detection of spores of *Nosema bombycis* and influence of recommended antiprotozoan drugs on fecundity and hatching of *Bombyx mori* L. were carried out during the year 2002 and 2003. The incidence of the disease was recorded in three different Sericultural zones of Kashmir valley viz., North, Central and South zone during June-July, 2002. The studies on the detection of the pathogen spores were carried out at Faculty of Agriculture, SKUAST(K), Wadura and the studies regarding the influence of drugs were carried out at the Division of Sericulture, SKUAST-K, Mirgund.

Observations of the present study revealed the prevalence of the pebrine pathogen in all the three Sericultural Zones at varied incidence. The disease was observed in various silkworms races viz., $SH_6\times NB_4D_2$, $NB_4D_2\times SH_6$, $CSR_4\times CSR_2$, $NB_4D_2\times$ KA, $NB_{18}\times SH_6$ and NB_4D_2 (P1) which were encountered during the course of study. The highest incidence of the disease (18.98%) was observed in South zone (Anantnag-Pulwama) and the lowest (9.00%) in Central

zone (Srinagar-Budgam). The infection was found to be due to secondary contamination as in case of transovarial type of infection the worms do not grow and survive beyond the III-instar.

Amongst the various methods which were undertaken for the detection of pebrine spore, maximum sedimentation of spores (13.48×10^5 ml^{-1}) was recorded when the centrifugation was carried out at 5000 revolutions for 5 minutes which was tested statistically at par with 5000 revolution for 4 minutes, 5000 revolution for 5 minutes, 5000 revolution for 2 minutes, 5000 revolution for 1 minute, 4000 revolution for 5 minutes, 4000 revolution for 4 minutes, 4000 revolution for 3 minutes and 4000 revolution for 2 minutes. The different stains which were utilized in the present study include: Giemsa, Delafield hematoxylin, Eosin, Safranin, Methyl blue, Malachite green, Sudan-III and Crystal violet. Out of these stains Safranin, Melachite green and Methyl blue effectively stained the spores where as Hematoxylin and Eosin gave better results when both these stains were applied to the same smear (Double staining).

All the chemicals/antiprotozoan drugs evaluated at different concentrations showed significant effect upon fecundity and hatching of the silkworm eggs when compared to the check-I and check-II. However, among themselves the chemicals/antiprotozoan drugs recorded uniform percent decline over control in both these characters when tested statistically. Ornidazole 0.4 per cent recorded the lowest fecundity of 535.73 eggs/ female and Chloroquine 0.4 per cent recorded the lowest hatching percentage to the extent of 79.22 per cent.

Plate 1: Rearing of Silkworms under optimal conditions temperature and relative humidity

Fig I: Rearing of silkworms inside the disinfected rearing room

Fig II. Silkworms in the rearing tray

Plate 2: Symptoms of Pebrine disease

Fig I: Silkworm larvae infected with *Nosema bombycis*

Fig II: Cocoons of Pebrine infected silkworm

Fig III: Silkmoths infected with *N. bombycis*

Plate 3: *Nosema bombycis* spores under microscopic field

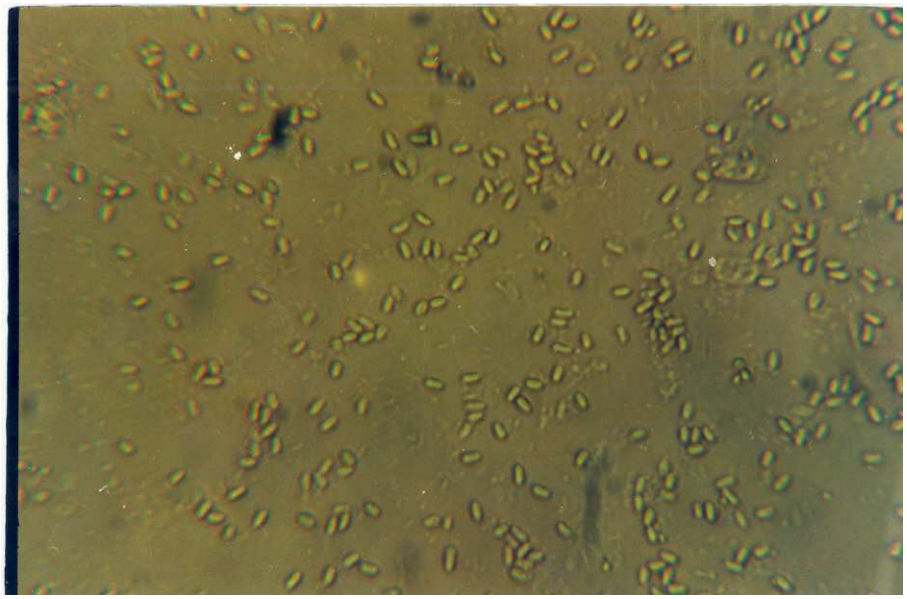


Fig I: ***Nosema bombycis*** **spores under 600x**

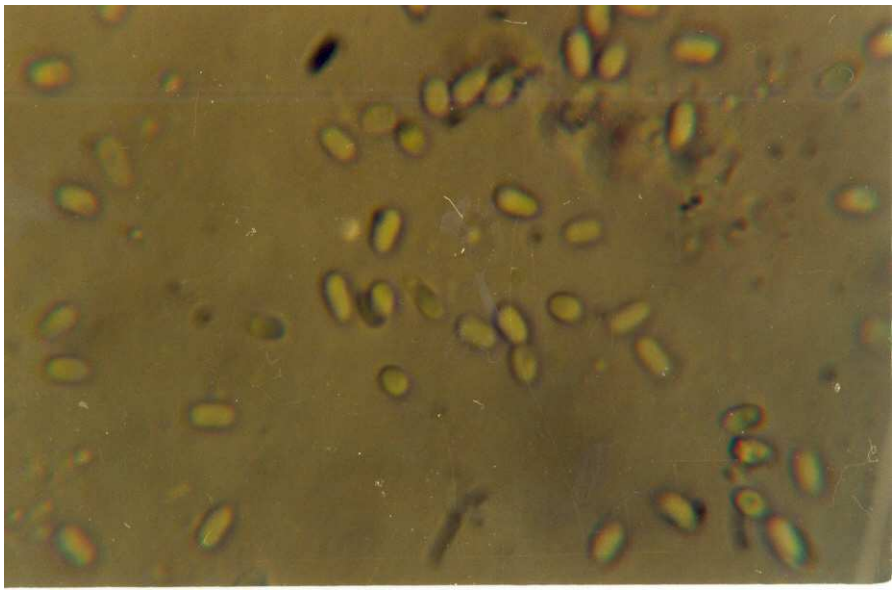


Fig II: ***Nosema bombycis*** **spores under 1500x**

Plate 4: Staining Studies

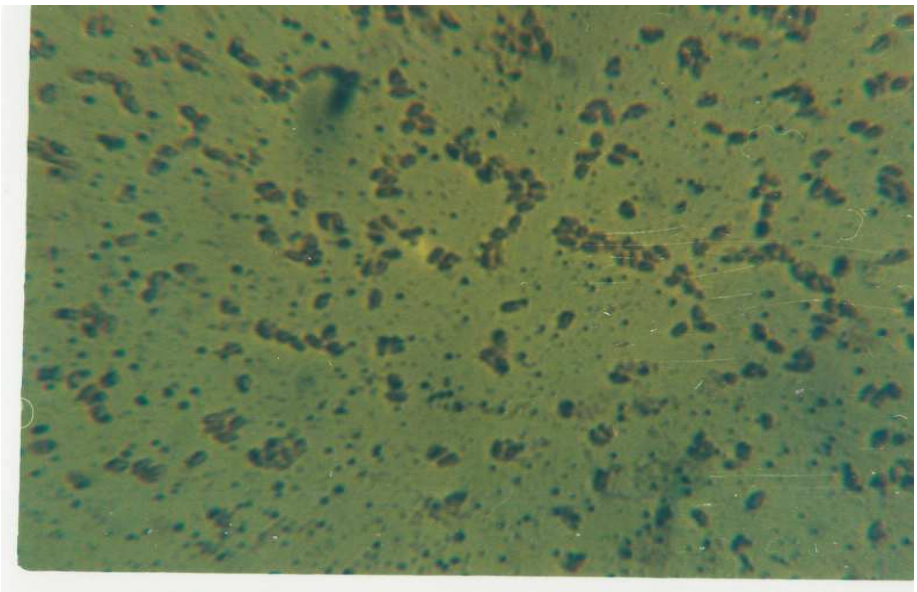


Fig I: Double stained (Hematoxylin-Eosin) spores of *N. bombycis* under 600x microscopic filed

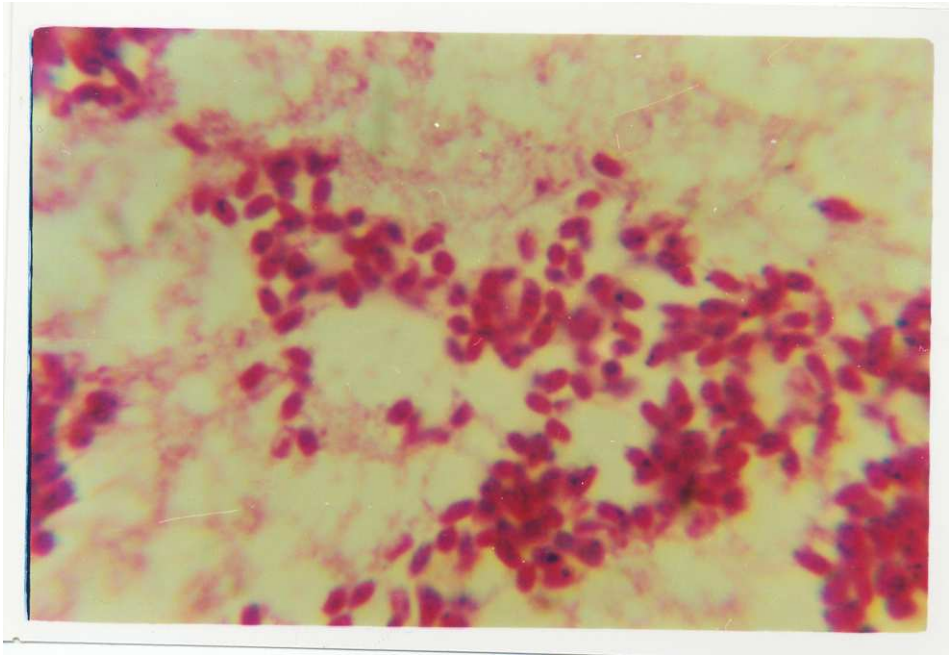


Fig II: Double stained (Hematoxylin-Eosin) spores of *N. bombycis* under 1500x microscopic filed

Plate 5: Staining studies

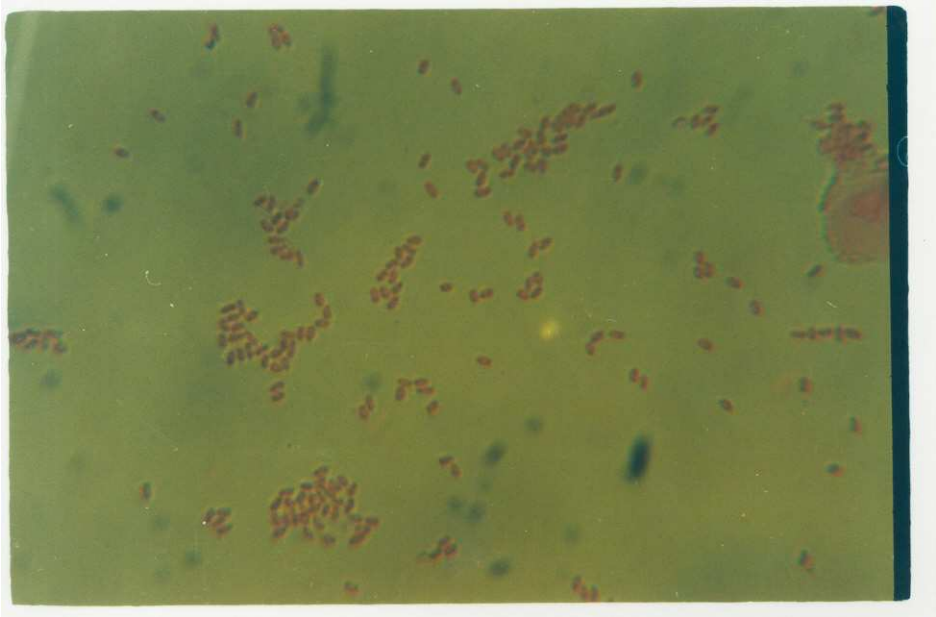


Fig I: Safranin stained spores of *N. bombycis* under 600x microscopic field

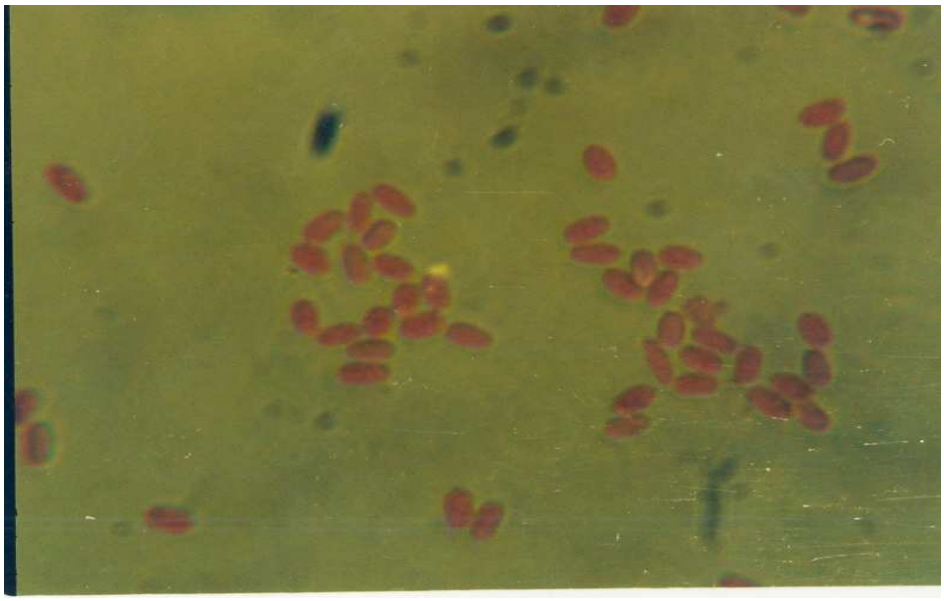


Fig II: Safranin stained spores of *N. bombycis* under 1500x microscopic field

Plate 6: Staining studies

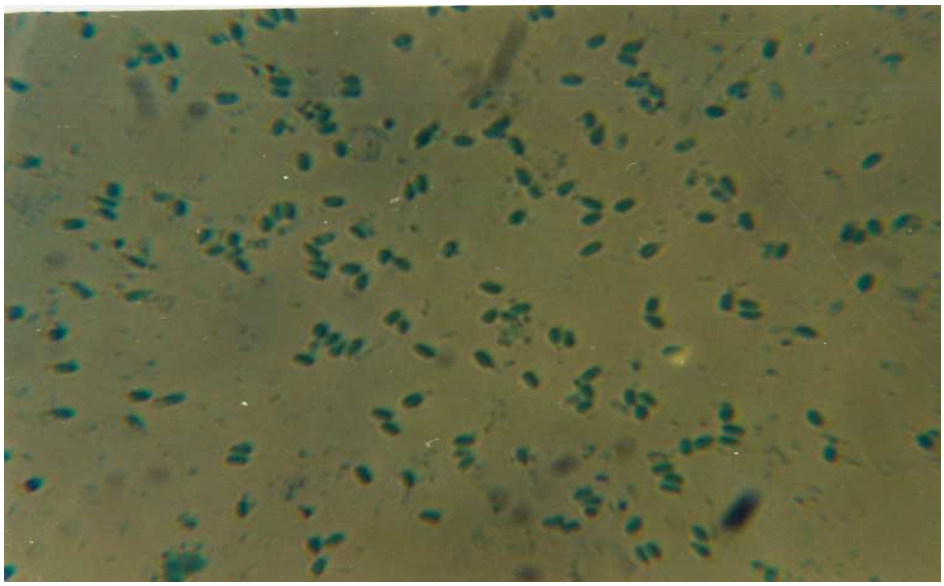


Fig I: Malachite green stained spored of *N. bombycis* under 600x microscopic field

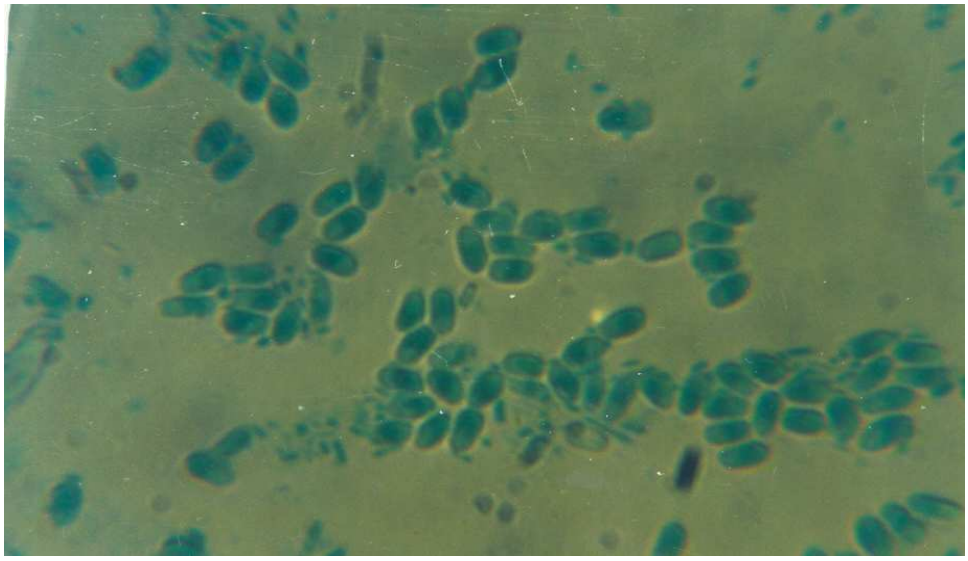


Fig II: Malachite green stained spored of *N. bombycis* under 1500x microscopic field

Plate7: Staining studies

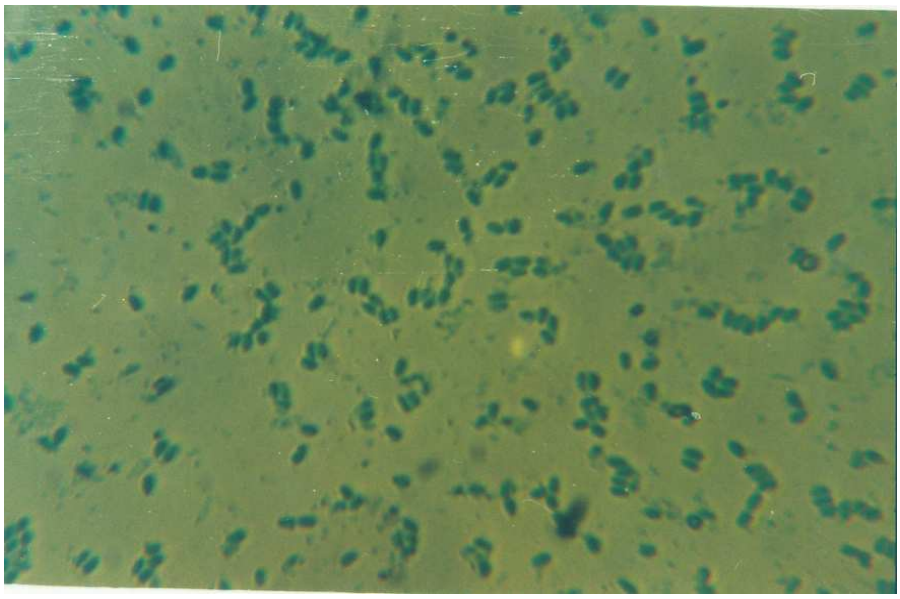


Fig I: Methyl blue stained spored of *N. bombycis* under 600x microscopic field

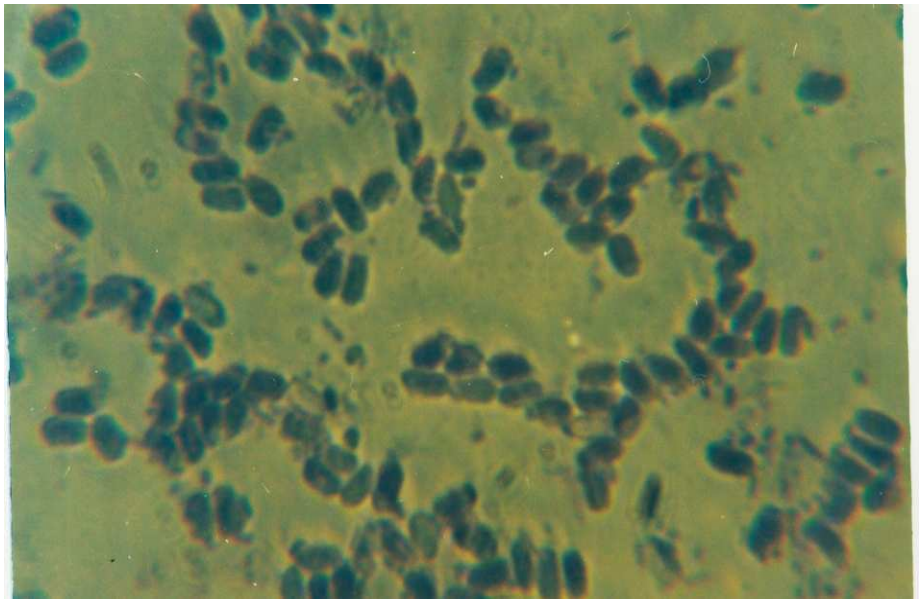


Fig II: Methyl blue stained spored of *N. bombycis* under 1500x microscopic field

Plate 8: Staining studies

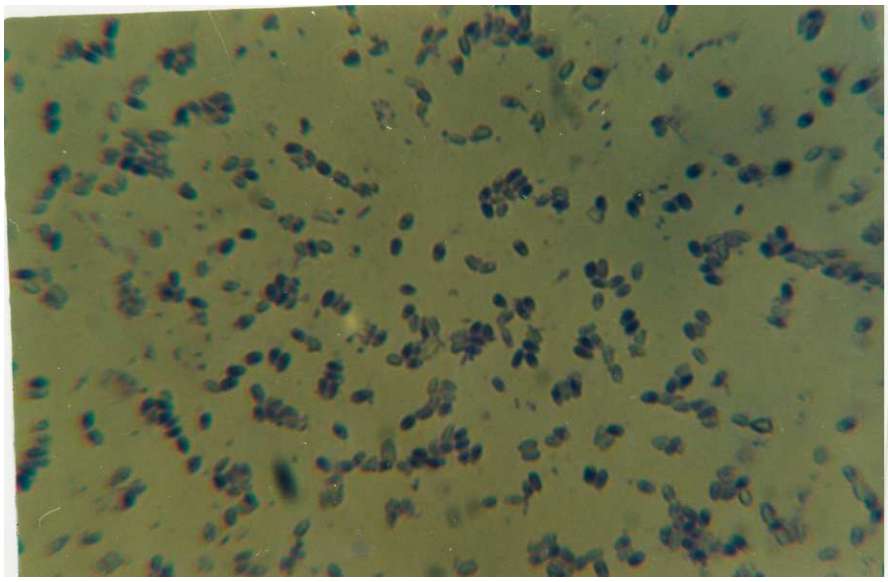


Fig I: Crystal Violet stained spored of *N. bombycis* under 600x microscopic field

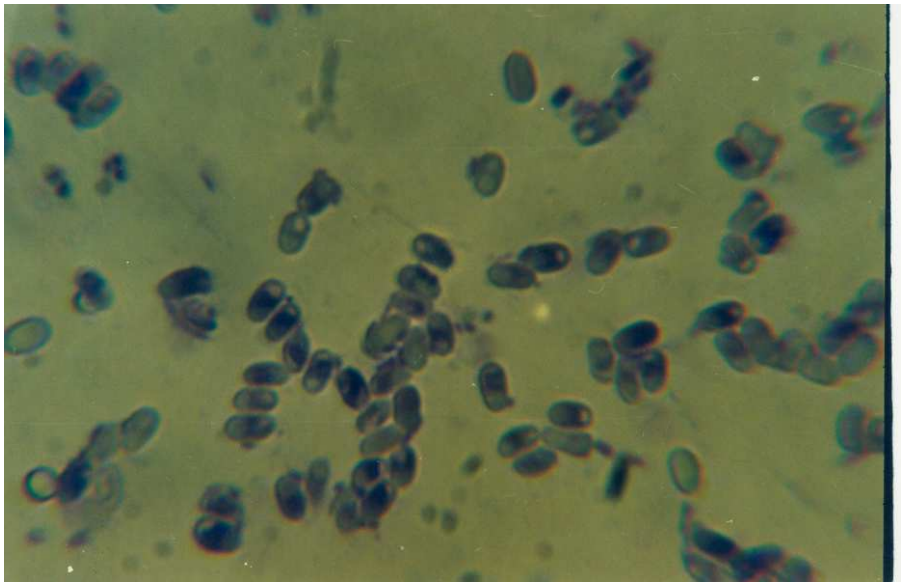


Fig II: Crystal Violet stained spored of *N. bombycis* under 1500x microscopic field

Plate 9: Staining studies

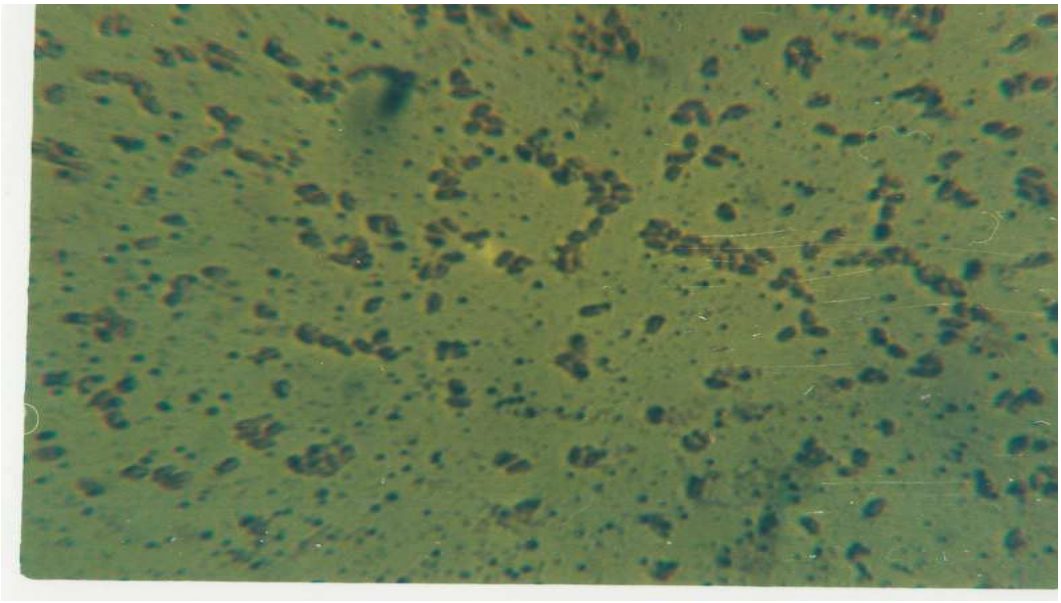


Fig I: Giemsa Violet stained spored of *N. bombycis* under 600x microscopic field

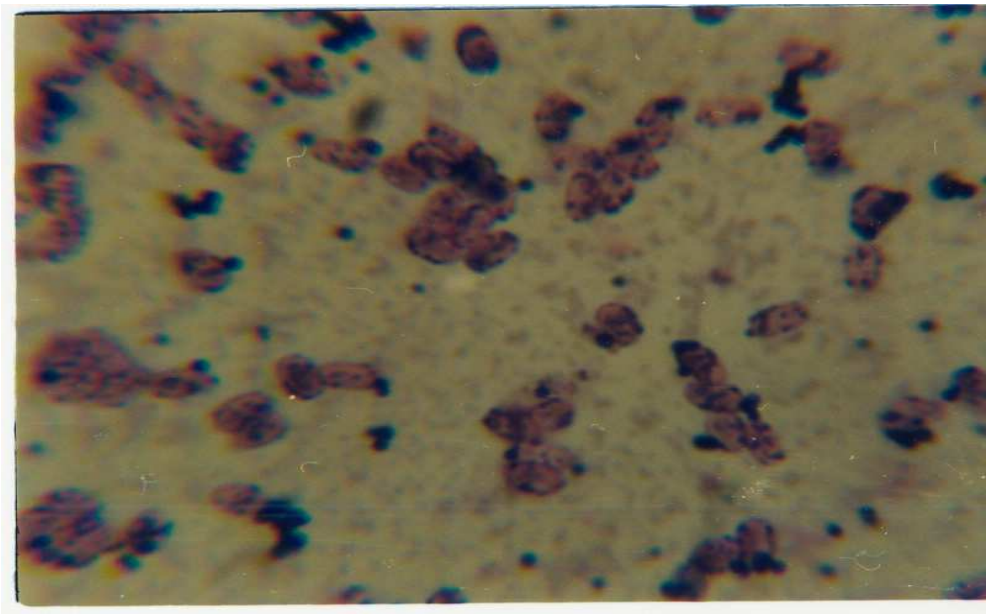


Fig II: Giemsa Violet stained spored of *N. bombycis* under 1500x microscopic field

LITERATURE CITED

Ananthalakshmi, K. V. V., Samson, M. V., Baig, M. and Datta, R. K. 1994b. The development stages and site of infection of *Nosema bombycis* in silkworm, *Bombyx mori* L. *Indian Journal of Sericulture* **33**(2): 180-181.

Anonymous, 1981. Silkworm Disease-I. Pebrine: causes and prev*ention*. *Research Bulletin No. 1,* Directorate of Sericulture Research, Ministry of Industries and Commerce, Kashmir p.1-18.

Anonymous, 1995. Incidence of pebrine in seed cocoons. *Annual Report 1994-95.* Central Silk Board, Madiwala, Banglore. p. 54-55.

Anonymous, 1996b. Effect of Albendazole against pebrine infection in silkworm. *Annual Report 1995-96*. Central Sericultural Research and Training Institute, Berhampore, p. 88-105.

Anonymous, 1997. Note for CSB meeting for development of Sericulture in Jammu and Kashmir State SKICC, Srinagar.

Anonymous, 1999. Technical document Directorate of Sericulture, Kashmir.

Anonymous, 2000a. "*Asian Textile Journal*", August: 46-47.

Anonymous, 2002. *Digest of Statistics (2001-2002).* Directorate of Economics Statistics Planning and Development Department Jammu and Kashmir p. 176-178.

Anonymous, 2003. Silk production in India to rise by 15 per cent per annum. *Textile Express*, 23rd March, 2003.pp.2.

Baig, M., Nataraju, B. and Samson, M. V. 1990. Studies on the spread of disease in the rearings of silkworm, *Bombyx mori* L. through different sources of contamination. *Indian Journal of Sericulture* **29**(1): 145-146.

Balavenkatasubbaiah, M., Sasidharan, T. O., Nageswara Rao, T., SelvaKumar, T. and Samson, M. V. 1992. Incidence of silkworm disease in Sericultural areas in Karnataka. *Annual Report 1991-91*. Central Sericultural Research and Training Institute, Mysore. p.54.

Bansal, A. K., Sinha, B. R. R. P., Sexena, N. N., Shukla, R. M., Roy, D. K. and Sinha, S. S. 1996. Control measures against microsporidiosis. *Annual Report 1995-96.* Central Tasar Research and Training Institute, Ranchi p. 47.

Bansal, A. K., Sexena, N. N., Shukla, R. M., Roy, D. K., Sinha, B. R. R. P. and Sinha, S. S. 1997. A new technique proposed for estimation of pebrine in Grainages. *Sericologia* **37**(1): 11-14.

Chandra, A. K. and Saha Kundu, A. K. 1982-83. The effect of drug on pebrine infection in *Bombyx mori* L. *Indian Journal of Sericulture* 21-22: 67-69.

Chandra, A. K., Saha, R. K., Bhattacharya, J., Krishnan, N., Sen, S. K. and Saratchandra, B. 1995. Efficacy of carbendazim as an antimicrosporidial agent and its influence on the growth and cocoon characters of silkworm, *Bombyx mori* L. *Insect Sciences and its Application*, **16**(2): 233-235.

Chitra, C., Karanth, N. G. K. and Vasantharajan, V. N. 1975. Diseases of the mulberry silkworm, *Bombyx mori* L. *Journal of Scientific and Industrial Research* **34**: 386-401.

Dadas, M. E., KhatavKar, R. S. and More, N. K. 1991. Comparative study of haemocyte population in scorpions. *Entomon* **16**(2): 159-161.

Dar, H. U. and Singh, T. P. 1998. Improved rearing techniques for *Bombyx mori* L. in Jammu and Kashmir. *Oriental Science.* **3**: 30-41.

Devaiah, M. C. and Krishnaswamy, S. 1975. Observations on the seasonal incidence of pebrine disease of silkworm, *Bombyx mori* L. *Indian Journal of Sericulture* **14**(1): 27-30.

Fujiwara, T. 1993. "Pebrine disease and its Control" *Proceedings of the trainers training programme* April 26 and 27. Central Sericultural Research and Training Institute, Mysore, p.1-44.

Geetha Bai, M., Patil, C. S. and Kasturi Bai, A. R. 1985. A new method for easy detection of pebrine spores. *Sericologia* **25**(3): 297-300.

Govindan, R., Narayamswamy, T. K. and Devaiah, M C. 1997. "Pebrine disease of silkworm", *Bulletin* University of Agricultural Sciences GKVK, Banglore-560065, pp.1.

Griyaghey, U. P., Sengupta, K., Kumar, P., Murti, R., Sinha, M. K. and Sinha, U. P. S. 1987. Studies on the effectiveness of Bengard in controlling the microsporidian disease of *Antherea mylitta* D. *Sericologia* **27**: 533-540.

Griyaghey, U. P., Murti, R., Kumar, V., Quadir, S. M., Kumar, A. and Sinha, S. S. 1989b. Control measures against microsporidiosis of *Anthereae mylitta. Annual Report 1988-89*. Cental Tasar Research and Training Institute, Ranchi p. 38-47.

Han, M. S. and Watanabe, H. 1988. Transovarial transmission of two microsporidian in the silkworm, *Bombyx mori* and disease occurrence in the progeny population. *Journal of Invertebrate Pathology* **51**(1): 41-45.

Hayashiya, S. and Ayuzawa, C. 1987. Diagnosis of microsporidians, *Nosema bombycis* and closely related species by antibody sensitized latex. *Journal of Sericultural Sciences,* Japan **52**(2): 169-170.

Jolly, M. S., Samson, M. V., Baig, M. and Nataraju, B. 1981a. Incidence of silkworm diseases in Karnataka. *Annual Report 1980-81.* Central Sericultural Research and Training Institute, Mysore, p 113-118.

Jolly, M. S., Samson, M. V., Nataraju, B., Baig, M. and Rani, B. A. K. 1981b. Effect of two antiprotozoan drugs on pebrine disease in silkworm. *Annual Report 1980-81*. Central Sericultural Research and Training Institute, Mysore, p 113-115.

Jolly, M. S. 1986. "Pebrine and its Control" Sericultural Research and Training Institute, Mysore, *Bulletin* **5**: 1-34.

Kamili, A. S., Masoodi, M. A. 2000. *Principles of Temperate Sericulture*. Kalyani Publishers p. 20,163.

KashKarova, L. F. and Khakhanova, A. I. 1980. Range of hosts of the agent of microsporidiosis (*Nosema bombycis)* of the Chinese silkworm. *Parasitologiya* (Leningr.) **14**(2): 164-167.

Lawrence, W. R. 1967. "*The valley of Kashmir*". Kesar Publishers, Kashmir p. 367-369.

Liu Shi Xian. 1987. The effect of chemotheraphy on pebrine disease of *Bombyx mori. Sericologia* **27**(3): 405-410.

Mariswamy, N. and Saikia, H. C. 1977a. Assessment of losses of crop due to different diseases in Muga and Eri silkworm. *Annual report 1976-77* Central Muga and Eri Research Station. Central Silk Board Govt. of India Titabar (Assam) p. 25.

Naegeli, 1857. "Pebrine Disease of silkworm". A Technical report (Ed: Tatsuke, K.) 1971, overseas Technical Cooperation Agency, Tokyo Japan.

Noamani, R. K., Krishnaswami, S. and Saha Kundu, A. K. 1971a. Assessment of different cocoon characters of the multivoltine silkworm (*Bombyx mori* L.) as influenced by pebrine disease. *Indian Journal of Sericulture* **10**(1): 96-100.

Noamani, R. K., Krishnaswami, S., Saha Kundu, A. K. 1971b. Observations on the seasonal incidence and intensity of pebrine disease of the silkworm (*Bombyx mori* L.) under west Bengal Climatic Conditions. *Indian Journal of Sericulture* **10**(1): 101-104.

Patil, C. S. and Geetha bai. 1989. Studies on the susceptibility of silkworm races to pebrine spore, *Nosema bombycis* Naegeli. *Journal of Applied Entomology* **108**(4): 421-423.

Prasad, N. R., Govindharaju, S. T. and Keshawa Reddy, K. S. 2000. Positive staining method for detection of pebrine spore, *Nosema bombycis* in silkworm, *Bombyx mori* L. *Sericologia* **40**(4): 575-580.

Quadrefagues, A. DE. 1860. Cited in Pebrine Disease of Silkworm-A Technical report (Ed.) Tatsuke, K.) 1971, OTCA Tokyo Japan.

Rao, M. M. 1998. A text book of Sericulture, BSP Publication. p. 136-138.

Rekha, L., Nataraju, B. and Swaprasad, V. 1998. Studies on group moth inspection method for production of Pebrine free layings in commercial seed production centres. *Indian Journal of Sericulture* **37**: 148-153.

Saha Kundu, A. K. and Chandra, A. K. 1978. Studies on the effect of Benomyl and Mebandazole on Pebrinised Silkworm larvae and its control. *Annual Report (1977-78*). Central Sericultural Research Station Berhampore (West Bengal) p. 31-33.

Saha Kundu, A. K. and Mondal, K. C. 1975. Studies on the Pebrine infection in eggs from female Pebrinised moth. *Annual Report 1974-75*. Central Sericultural Research Station Berhampore (West Bengal) p. 50-51.

Saha Kundu, A. K. and Mustafi, J. C. 1980. Pebrine disease in silkworm and its control. Annual Report 1979-80. Central Sericultural Research Station Berhampore (West Bengal) p. 31-36.

Samson, M. V., Baig, M., Sharma, S. D., BalavenKatasubbaiah, M., Sasidharan, T. O. and Jolly, M. S. 990. Survey on the relative incidence of silkworm disease in Karnataka India. *Indian Journal of Sericulture* **28**(2): 248-254.

Sato, R., Kobayashi, M., Watanabe, H. and Fujiwara, T. 1981. Serological discrimination of several kinds of microsporidian spores isolated from the silkworm, *Bombyx mori* L. by an indirect fluorescent antibody technique. *Journal of Sericultural Sciences* Japan **50**: 180-184.

Sato, R. and Watanabe, H. 1980. Purification of mature microsporidian spores by isodensity equilibrium centrifugation. *Journal of Sericulture Sciences* Japan **49**: 512-516.

SelvaKumar, T., Mary Flora, C. A., Krishore, S. and Nageswara, Rao, S. 1994. Incidence of silkworm disease in Sericulture areas of Karnataka. *Annual Report 1993-94.* Central Sericultural Research and Training Institute, Mysore, p. 99-100.

Sengupta, K., Baig, M., Samson, M. V. and Nataraju, B. 1993. Immuno-diagnosis of the Pebrine disease in silkworm. *Sericologia* **33**(1): 147-149.

Sprague, V. 1977. The Zoological distribution of microsporidia *In*: *Comparative Pathobiology* (Vol. II) L. A. Bulla and T. C. Cheng (Eds.), Plenum Press, New York, p 510.

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

Talukdar, J. N. 1974. Silkworm diseases and pests. *Annual Report 1973-74*. Central Muga and Eri Research Station, Central Silk Board Titabar.

Watanabe, H. 1987. Diagnosis of pebrine through antibody sensitized latex. *Journal of Sericultural Sciences* Japan **56**(5): 431-435.

Zargar, M. A. 2001. Studies on the pathogencity of *Nosema bombycis* Naegeli to silkworm, *Bombyx mori* L. and its management, Ph. D Thesis of Division of Entomology (SKUAST-K) Jammu and Kashmir.

Zargar, M. A., Gaffar, S. A., Kamili, A. S., Masoodi, M. A., Munshi, N. A.,Tanki, T. N. and Dar, H. U. 2002. Distribution and incidence of Pebrine disease in various commercial races/hybrids of silkworm, *Bombyx mori* L. in Kashmir. *Applied Biological Research* **4**: 39-42.

Appendix-I

1. Incidence of pebrine disease (*Nosema bombycis* N.) of mulberry silkworm (*Bombyx mori* L.) in various Sericultural Zones of Kashmir valley

i) Zone-Wise

S.V.	DF	SS	MSS	F ratio	F prob.
Replication	**4**	**161.95**	**40.49**	**1.28**	**0.353**
Treatment	2	250.71	125.35	3.97	0.064
Error	8	252.72	31.59		
Total	14	665.38			

---

ii) Location-wise

Replication	**4**	**1032.8**	**258.2**	**1.90**	**0.116**
Treatment	27	6784.3	251.3	1.85	0.014
Error	108	14675.4	135.9		
Total	139	22492.5			

Appendix-II

2. Centrifugation

i) Influence of different centrifugation speeds and durations on sedimentation concentration of pebrine spores (*Nosema bombycis* N.)

S.V.	DF	SS	MSS	F ratio	F prob.
Treatment	**25**	**557.312**	**22.292**	**63.95**	**0.000**
Error	52	18.127	0.349		
Total	77	575.439			

iii) Improvement in sedimentation concentration of pebrine spores (*Nosema bombycis* N.) over control

S.V.	DF	SS	MSS	F ratio	F prob.
Revolutions	**4**	**2446.63**	**611.664**	**58.28**	**0.000**
Duration	4	107.86	26.96	20.20	0.000
Rev.×Durations	16	72.94	4.56	3.42	0.000
Error	50	66.73	1.33		
Total	74	2694.16			

Appendix-III

3. Influence of some recommended antiprotozoan drugs on fecundity (eggs/female) and hatching percentage of *Bombyx mori* L.

i) Fecundity

S.V.	DF	SS	MSS	F ratio	F prob.
Treatment	**19**	**20831.8**	**1096.47**	**59**	**0.000**
Error	40	5781.8	144.5		
Total	59	26613.5			

---

ii) Hatching percentage

Treatment	**19**	**747.73**	**39.35**	**3.60**	**0.000**
Error	40	437.12	10.93		
Total	59	1184.85			

Appendix-IV

4. Percent decrease in fecundity

i) Over Check-I

S.V.	DF	SS	MSS	F ratio	F prob.
	Treatment	**5**	**224.6544.93**	**2.24**	**0.071**
Concentration	2	266.10	133.05	6.64	0.003
Treatment× Concentration	10	45.18	4.52	0.23	0.992
Error	36	720.84	20.02		
Total	53	1256.77			

ii) Over Check-I I

S.V.	DF	SS	MSS	F ratio	F prob.
	Treatment	**5**	**159.0031.80**	**2.18**	**0.077**
Concentration	2	195.13	97.56	6.70	0.003
Treatment× Concentration	10	35.26	3.53	0.24	0.989
Error	36	524.04	14.56		
Total	53	913.43			

Appendix-V

5. Percent decrease in hatching percentage

i) over check-I

S.V.	DF	SS	MSS	F ratio	F prob.

Treatment	**5**	**106.88**	**21.38**	**0.72**	**0.612**
Concentration	2	266.69	133.35	4.49	0.018
Treatment× Concentration	10	89.16	8.92	0.30	0.976
Error	36	1068.33	29.68		
Total	53	1531.06			

ii) over check-II

S.V.	DF	SS	MSS	F ratio	F prob.
Treatment	**5**	**64.82**	**12.96**	**0.55**	**0.740**
Concentration	2	125.70	62.85	2.65	0.085
Treatment× Concentration	10	36.12	3.61	0.15	0.998
Error	36	854.73	23.74		
Total	53	1081.38			