Management of Pebrine Disease in Bivoltine Silkworms Reared under Temperate Climatic Conditions

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(2017-S-52-M)

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Thesis

Submitted to

Sher-e-Kashmir
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in partial fulfilment of requirements for the award of the degree of

Master of Sciences in Sericulture

2020


Dedicated To My
Beloved
Parents

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
College of Temperate Sericulture, Mirgund, Baramulla

Certificate – I

This is to certify that the thesis entitled, **"Management of Pebrine Disease in Bivoltine Silkworms Reared under Temperate Climatic Conditions"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Sciences in Sericulture** to the **Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, College of Temperate Sericulture** is a record of bonafide research work carried out by **Ms. Arbia Fatima (Reg. No. 2017-S-52-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 information received during the course of investigation has duly been acknowledged.

(Prof. Afifa S. Kamili)
Chairperson
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We, the members of the Advisory Committee of **Ms. Arbia Fatima (Reg. No. 2017-S-52-M)**, a candidate for the degree of **Master of Sciences in Sericulture** have gone through the manuscript of the thesis entitled, **"Management of Pebrine Disease in Bivoltine Silkworms Reared under Temperate Climatic Conditions"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of degree.

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This is to certify that the thesis entitled, **"Management of Pebrine Disease in Bivoltine Silkworms Reared under Temperate Climatic Conditions"** Submitted by **Ms. Arbia Fatima (Reg. No. 2017-S-52-M)** to the **Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, College of Temperate Sericulture** in partial fulfilment of the requirements for the award of the degree of **Master of Sciences in Sericulture** was examined and approved by the Advisory Committee and External Examiner on ……..

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ABSTRACT

Pebrine caused by *Nosema sp.* is a dreadful disease of silkworm, *Bombyx mori. L* and has ruined the silk industry in France and Itlay as early as in 1845. The disastrous effect of pebrine was even seen In Kashmir by the loss of its productive indigeneous univoltine breed "Kashmir Race" with the outbreak of this dangerous disease 131 years back. The disease can cause 100 per cent loss of cocoons and enormous economic loss to the farmers if not managed properly. Keeping in view the magnitude of this disease as well as value of sericulture in the current times it was seriously thought to work out a sound and cost effective method of managing this dreadful disease. This led to the conception of idea of studying effect of different chemicals for the management of the disease. The present findings reveal that out of three chemicals screened for their efficacy against the disease Sodium bicarbonate was found most effective as compared to Ornidazole and Rifampicin. Among the various treatment combinations Sodium bicarbonate at 0.8 per cent concentration performed significantly better with almost all the parameters under study *viz*., Larval survival (87.39 per cent), Weight of ten mature larvae (51.46 g), Cocoon yield by number (8550), Cocoon yield by weight (19.92 kg), Percentage of live cocoons (97.80 per cent), Average single cocoon weight (2.39 g), Average single shell weight (0.43 cg), Shell percentage (19.66), Moth emergence percentage (95.93 per cent), Recovery percentage of dfls (96.31 per cent), Average single filament length (1205 m).

The study gave an idea that Sodium bicarbonate at 0.8 per cent can be used for the management of the pebrine disease which inturn will help the farmers to improve their economic returns. Besides Sodium bicarbonate has an added advantage of being readily available and cost effective.

Key words: Pebrine, Silkworm, Management, Chemicals.

Signature of Student

Dated:_____

Signature of Major Advisor

Dated:_____

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Arbia Fatima

Place: Mirgund

Dated:

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Chapter 1

INTRODUCTION

In India, sericulture is not only a tradition but also a living culture. Indian silk has enthralled all categories of consumers across the world with its vast repertoire of motifs, techniques and brilliant hues. Silk reigns supreme as an object of desire, famous for its fabric of high fashion, its finery and distinct colours. Mankind has always loved the shimmering fibre of unparalled grandeur from the moment Chinese Empress Si Ling-chi discovered it in her tea cup. It withstood many daunting challenges from other natural and artificial fibres and yet remained the undisputed "Queen of Textiles" till date. Having a rich and complex history in silk production and silk trade that dates back to 2nd century B.C India enjoys the status of being the only country in world with richest seri-biodiversity. The base of Indian silk industry is associated with the traditional states like Jammu & Kashmir West Bengal, Karnataka, Andhra Pradesh and Tamil Nadu. These five states collectively account for 95% of the total area under mulberry cultivation and 92% of the raw silk production in the country. Though sericulture occupies a unique position in Indian economy and assumes more importance in alleviating the problems of rural people, it is highly suitable in the context of diversification of farm enterprises and integration of farming systems with other enterprises to generate attractive income (Prakash and Dandin, 2005).

Out of the total 6,38,588 villages in India, sericulture is practiced in about 52,360 villages (Bharathi, 2016). The estimated employment generation during the year 2017 was about 8.60 million persons. The total area under mulberry in India amounts to 2.40 lakh hectares with a raw silk production of 14,257 MT (Anonymous, 2018).

Jammu & Kashmir state has been one of the trailblaizer states for the production of univoltine/bivoltine silk of international standard. At present, sericulture is practised in 20 districts of the state. Among them, major silk

producing districts include Anantnag, Kupwara, Pulwama, Baramulla, Ganderbal, Udhampur, Rajouri, Reasi and Kathua. The raw silk production in Jammu & Kashmir was 190 MT in the year 2018 (Anonymous, 2018).

The mulberry silkworm, *Bombyx mori* L., since times immemorial has been the prey of various dreadful diseases such as pebrine, muscardine, grasserie and flacherie. In India, the average silkworm cocoon crop loss due to various diseases is to the tune of 15-47% (Tayal *et al.*, 2017). Among these diseases, the pebrine, caused by *Nosema sp.* is one of the most dreadful diseases having potential to wipe out the silk industry.

In the state of Jammu & Kashmir for hundreds of years, silkworms and its seeds were free from any infestation especially pebrine and the state was considered the disease free silkworm seed producing state in the whole world so much so, that in France in the year 1845 when silkworms were attacked by the dreadful disease "Pebrine" the silk industry there got devastated. It was the state of Jammu & Kashmir that came to their rescue by supplying 25,000 ounces of disease free silkworm seeds of its indigenous race popularly known as "Kashmir Race", to France to revive the silk industry there. But unfortunately, in due course of time silkworms in our state were also attacked by the same dreadful disease the "pebrine" scientifically known as Microsporidiosis . It is due to the outbreak of Microsporidiosis that Kashmir lost its only productive Univoltine silkworm race "Kashmir Race" in 1878 (Kamili and Masoodi, 2000). Since then J&K state became dependent on silkworm seeds imported from different sericultural countries and states. The disease has become more complex now because of the perpetual incidence of different types of microsporidian infection in silkworms (Sharma *et al.,* 2003). Recently, the incidence of Microsporidiosis occurrence in Kashmir Division has been reported to be 18.98%, 13.46%, 9.00% in South, North & Central zones of Kashmir, respectively (Ganie *et al.,* 2008).

The pebrine disease is transmitted by spores of the disease called microsporidia. The microsporidia are microscopic spores, intracellular living

eukaryote infecting both beneficial and non-beneficial insects (Nataraju *et al.*, 2005). More than 140 genera and 1200 species of microsporidia have been recorded from insects and fish (Samson *et al.*, 1999). Among these, at least 200 belong to the genus Nosema (Sprague, 1982) and majority of these, including *Nosema bombycis* is pathogenic to silkworms, *Bombyx mori* L. (Canning *et al.*, 1999). The lifecycle of *Nosema bombycis* includes three stages, namely, spore, plannot and meront. The mature spore is oval and measures approximately 3.4-3.8 µm in length and 2.0-2.3 µm in width. The spores are highly refractile and shine bluish white under microscope exhibiting 'Brownian movement'.

The pebrine disease starts with the ingestion of spores through contaminated mulberry leaf. After digestion, spore wall gets dissolved in the gut and sporoplasm creeps out and enters the gut wall. The sporoplasm multiplies through fission and spreads to every part of the body in the form of a spore (Abe & Fujiwara, 1979). The spore completes its life cycle in one week under temperate conditions and 4days under tropical climatic conditions (Kamili & Masoodi, 2000). The infected larvae become sluggish and show symptoms like poor appetite, retarded growth and development with irregular moulting. With the progress of this disease, the larvae become pale, dull & translucent with wrinkled skin, shrink in size and become flaccid (Singh & Sarat Chandra, 2003) and ultimately die before the onset of 3rd moult with the high spore load (Singh *et al.*, 2010). However, some worms with the lesser spore load may survive but become the carriers of infection. The infection either spreads by transovarial transmission or by horizontal transmission. Transovarial transmission has been 100% in the case of *Nosema bombycis* (Han & Watanabe, 1988) thereby, causing total loss to the farmers.

Keeping in view the magnitude of this disease and the loss it causes to the farmers as well as value and importance of sericulture in the current times, it was seriously thought to work out a sound and cost effective method of managing this dreadful disease. Though some chemicals have been tried earlier against the

pebrine disease but the significant or total control over the disease has not been reported (Singh *et al.*, 2010), thus putting the silk industry of the state under the threat. Based on these facts, a study was proposed on the "Management of Pebrine Disease in Bivoltine Silkworms Reared under Temperate Climatic Conditions" with the following objectives:

1. To study the effect of some low cost chemicals on the management of silkworm microsporidiosis.
2. To study the effect of these chemicals on various economic parameters of the silkworms.
3. To identify the most suitable and cost effective chemical against the microsporidiosis for the benefit of farmers.

Chapter 2

REVIEW OF LITERATURE

Since literature available on the present study "Management of Pebrine Disease in Bivoltine Silkworms Reared under Temperate Climatic Conditions" is Scanty, an attempt has been made to review the literature pertaining to protozoan diseases attacking silkworm *Bombyx mori* L. and also related insects and other living creatures. The literature reviewed has been presented under the following headings:

2.1 Incidence of pebrine disease in silkworms

Krishnaswamy *et al.* (1978) reported the occurrence of pebrine disease in 20 silkworm races during all the rearing seasons under Mysore climatic conditions. They reported that variation in the percentage of infection among the silkworm races in different seasons. The percentage of infection was the highest during September-October (38.97%), followed by March (32.58%), November-December (14.95%), June-July (6.05%) and the lowest in April-May (4.66%). Authors have reported a minimum incidence of the pebrine disease during the months of April to July, when the temperature and the humidity were relatively higher and maximum incidence was reported from July to March, when the temperature and humidity were relatively low.

Chitra *et al.* (1975) carried out studies on seasonal incidence of pebrine in silkworms and reported that pebrine spreads faster in cool and humid atmosphere than under hot and dry conditions.

Jolly *et al.* (1981a) have reported 6.95% incidence of pebrine infection from the cocoon samples of silkworms collected in five months from different cocoon markets located in different regions of Karnataka.

Jolly (1986) reported that the incidence of pebrine is more severe during summer season followed by autumn and spring in temperate climatic conditions.

Selva Kumar *et al.* (1993) reported 36.34, 65.54, 57.18 and 19.88% of pebrine disease in mulberry silkworm during 1990, 1991, 1992 and 1993, respectively. The prevalence of the disease was seen highest during winter.

Sahaf (2002) reported that the annual loss due to microsporidiosis in silkworms in the state Jammu and Kashmir accounts upto 5%.

Zargar *et al.* (2002) carried out his survey studies on 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 District Anantnag followed by District Baramulla (46±2.0%), District Pulwama (44.1±2.5%), District Budgam (41.2±2.4%), District Kupwara (33.3±2.6%), and District Srinagar (25.4±2.1%).

The survey conducted by Ganie *et al.* (2008) stated that the incidence of microsporidiosis in silkworms in the Kashmir division ranged between 18.98, 13.46 and 9.00% in South, North and Central zones, respectively.

Kumar *et al.* (2011) reported that pebrine disease is considered to be the most serious disease in mulberry silkworm *Bombyx mori* L. and contributes 20-25% towards crop loss.

2.2 Techniques for Detection of Pebrine pathogens

Singh *et al.* (1992) suggested the surface sterilization of eggs immediately after egg laying and also during the pin head stage of incubation inorder to prevent the occurrence of pebrine disease in silkworms from the surface contamination of *Nosema bombycis* spores as the disease is seed born.

Hatakeyama and Hayasaka (2002) reported that *Nosema bombycis* are among the microsporidia that more frequently spread through transovarial transmission than horizontal transmission and they can be determined at the egg phase of the silkworm for the determination of the pebrine disease using PCR technique.

Hatakeyama and Hayasaka (2003) reported that the microsporidia in silkworm *Bombyx mori* L., can be diagnosed from the eggs of the silkworms using multiplex PCR technique, by examining the pieces taken directly taken from the larva or from the pieces of faeces using the centrifugal method of the spores.

Quadri and Khatri (2005) gave a three tier examination approach (larval, pupal and moth) to detect the incidence of pebrine disease in the multiplication of silkworm seed

Chakrabarty *et al.* (2013) reported about an improved method for detection of pebrine (*Nosema bombycis*) spores using powdered formulation of Ambistryn-S (antibiotic preparation of streptomycin sulphate) and Transco trichostar (a biofungicidal preparation of Trichoderma viridae) for larval, pupal and moth suspension after oviposition. This technique performed better in pebrine spore detection than other techniques even at very low infection rate in a small population.

2.3 Pathogenicity

Pathogenicity study conducted by Griyaghey and Kumar (1983) to find out the range in the number of *Nosema* spores present in the infected individuals of Tasar silkworm indicated that the number of spores in an individual moth ranged between $1.26\times10^{8}\mu m$ to $52.32\times10^{8}\mu m$. On an average the number of spores/individuals was found to be $7.67\times10^{8}\mu m$ irrespective of the sex.

Srikanta (1987) conducted the studies on cross-infectivity viability and pathogenicity of different isolates of *Nosema bombycis* on six lepidopterous insects and one dipterous maggot. The pathogen was found to readily infect *Corcyra cephalonica staint, Samia Cynthia ricini boisduval, Paruchaetes pseudoinserllactinea* Wlk*., Spilosoma oblique* Wlk*., Estigemens lactinea* Wlk*., Spodoptera litura* Hubner and *Heliothis armigera* Wlk. The pathogen did not infect any stage of the silkworm fly pest *Exorista sorbillan* Wiedmann. The cross infected lepidopterous insects showed sluggishness, uneven growth, loss of

appetite and difficulty in moulting. The viability studies on the *Nosema bombycis* spores revealed that the spores could remain viable even after 150 days of storage under refrigeration, upto 90 days at 15° C and room temperature under moist conditions. The spores stored at 35° C and 45° C were found to loose viability within 24 hours. At room temperature under dry conditions the spores loose viability within five days. Reduction in spore size was observed as the number of days of storing increased irrespective of the storage conditions. Isolates of Mandya district, Karnataka was found to be more virulent and caused significantly lowest single cocoon, pupa and shell weight as well as shell weight, shell ratio, filament length and denier than other isolates. Isolates from Kolar, Bangalore and Mysore districts were found to be less pathogenic.

Nageswara and Fujiwara (1994) conducted studies to examine the racial tolerance of pebrine disease between bivoltine and multivoltine silkworm races and found that none of the races possessed any racial difference of susceptibility to *Nosema bombycis*. However, an average life span of infected larvae after inoculation was observed as 16.1 days in NB_4 D_2, 16 days in NB_7, 15.8 days in NB_{18} (Bivoltine), 19 days in Pure Mysore, 14 days in C. Nichi and 15.7 days in Tamil Nadu white (multivoltine). These results indicated the presence of pre-racial difference with respect to tolerance towards pebrine disease in silkworms.

Pathogenicity tests conducted by Kishore *et al.* (1994) showed 100% transmission to *Bombyx mori* L. larva infected transovarially by *Nosema bombycis* spores.

2.4 Chemotherapy

Salim *et al.* (1972) carried out studies on rifampicin and observed that 1200mg/day rifampicin was able to cure the lesions of patients suffering from cutaneous leishmaniasis. He observed that 41 out of 46 patients were treated successfully from the protozoan disease after being treated with rifampicin.

Saha Kundu and Mustafi (1980) have reported about the anti-microsporidial acvtivity of Benomyl at Central Sericultural Research Station, Berhampore in West Bengal. The Benomyl treated leaves when fed to silkworms at 4000, 6000 and 8000 ppm resulted in reduction of larval mortality due to microsporidiosis and the chemical has been found effective towards the commercial cocoon characters in silkworms like cocoon weight, shell weight and effective rate of rearing.

Jolly *et al.* (1981b) observed that Fumidil-B and Bavistin when used at a concentration 1500-2000 ppm were effective to a large extent in managing the pebrine disease in silkworms. After the application of these anti-protozoan drugs no incidence of pebrine disease has been reported during the larval stage but in case of moth stage 62.53-80.1% of pebrine infection has been reported.

Baig *et al.* (1988) studied the comparative efficacy of four disinfectants (v.i.z; hilite, sodium hypochlorite, bleaching powder and formalin) at four concentrations i.e. 0.5, 1.0, 1.5 and 2% as surface sterilents against the spread of pebrine disease in a colony of silkworms hatched from the surface contaminated laying and reported that all the tested concentrations were effective in preventing the spread of the disease and also successful in inactivating the spores of *Nosema bombycis* when exposed to 5, 10, 20 and 30 minutes respectively.

Schmahl and Mehlhorn (1989) observed the effect of carbestin @0.01 & 0.1% concentration and pantelmin @ 0.1% concentration on *Nosema bombycis* and reported significant increase in effective rate of rearing, cocoon weight, shell weight and survival.

Liu (1990) observed the lethal effect on microsporidiosis due to antiprotozoal drugs viz., Emetine-Hcl and Formycin-B, Fumagillin in invitro conditions and reported 61% reduction in pebrine disease of silkworms.

Sichotva *et al.* (1993) reported about the anti-microsporidial activity of Singefungin, i.e, a natural nucleoside isolated from the *Streptomyces incarnatus*.

The chemical was reported highly effective against microsporidiosis (*Nosema apis*) in bees. The infection was eliminated from the midgut of young and over-wintering bees at a concentration of 4 ug/ml .

Balavenkatasubbaiah *et al.* (1994) while working on disinfectants like Bleaching powder @ 1, 2, 3, 4 and 5% concentrations and Formalin @2% concentration reported that these were effective in the inactivation of *Nosema bombycis* spores when exposed to the larvae in the rearing room for 10, 20 and 30 minutes at room temperature (25±1º C).

Chandra *et al.* (1995) have reported the efficacy of Bavistin (Carbendazium) at 2 and 3% concentration as antimicrosporidial agent in case of *Bombyx mori* L. The used treatments increased the survival of worms and reduced the pebrine infection. Besides, these chemicals have increased commercial cocoon characters like the cocoon shell weight and shell ratio.

Sheetz *et al.* (1997) studied the effect of rifampicin against Nosema species infecting Encarsia wasp (a parasitic protozoa). The drug @2.2% was found to decrease the level of microsporidian infection by 48.56% as compared to control where the infection rate was significantly high.

Kochar *et al.* (2000) reported that Rifampicin is an effective drug against protozoan disease Cutaneous Leishmaniasis in humans. He reported that 73.9% of the patients treated with rifampicin had complete healing.

Zargar (2001) studied the effect of antiprotozoan drugs viz., Bavistin, Topsin, Codrinal, Croydoxin-FM, Malariaquine and Metrogyl at various concentrations against pebrine disease of silkworm *Bombyx mori* L. and found that Bavistin resulted in 66.34% control over pebrine disease in silkworms.

Ozbilgin *et al.* (2002) carried out studies on the control of Giardiasis (a protozoan parasite) caused by *Giardia lamblia* and reported that 30mg/kg single dose of ornidazole was effective in treating 97% of patients suffering from giardiasis.

Boohene *et al.* (2003) studied the effect of the anti-protozoan drug rifampicin against *Nosema* disease in *Muscidifurax raptor* (an important biocontrol agent against filth flies). The antibiotic rifampicin @3% was found to decrease the rate of transmission of pebrine disease by 57.7%.

Goo *et al.* (2010) studied the effect of artesunate, a potential drug for the control of protozoan parasite *Babesia* spp. infected in mouse. The drug was administered @ 0.26, 2.6, 26, 260 µm artesunate in combination with 5% sodium bicarbonate. It was observed that 5% sodium bicarbonate in combination with 156 µm artesunate significantly suppressed the growth of *Babesia* species infecting mouse on day 9^{th}.

Bhat *et al.* (2012) reported that 1% concentration of ornidazole is effective in reducing the mortality due to pebrine by 96.46% in Lamerin breeds of silkworm & 92.68% in CSR_2 breeds of silkworm.

Santha *et al.* (2012) studied the effect of Decol (a disinfectant) for the management of pebrine disease in *Bombyx mori* ad reported that Decol is effective in controlling the pebrine disease in silkworms.

Peng *et al.* (2015) evaluated the effect of sodium bicarbonate on freshwater green alga *Neochloris oleobundans* which was inoculated with protozoan pre cultures. They reported that 160 or 200 mmol/L sodium bicarbonate completely inhibits the growth of microalga preying protozoa.

Sebastian and Elke (2015) observed that ornidazole has shown invivo efficacy against *Nosema bombycis* in silkworm larvae *Bombyx mori* L. and resulted in 80-90% pebrine disease reduction when applied orally at 2.5 mg/ml upto 10 mg/ml.

Chapter 3

MATERIAL AND METHODS

The present investigation titled "Management of the Pebrine Disease in Bivoltine Silkworms Reared under Temperate Climatic Conditions" was carried out in the year 2018 (Spring season) at KVK, Ganderbal, SKUAST-Kashmir. The study was carried out in two parts to fulfill the objectives:

- To study the effect of chemicals on the management of pebrine disease.
- To study the effect of chemicals on the commercial cocoon characteristics of the silkworms.

3.1 Material for study

3.1.1 Bivoltine silkworm race-SKUA-R 6

The silkworm seed of race SKUA-R 6 laid by the moths infected with pebrine (*Nosema*) were selected for the study programme.

3.1.2 Chemicals used for the management studies

1. Sodium bicarbonate @ 0.6%, 0.7% and 0.8% (T_1)
2. Ornidazole @ 0.75%, 1.0% and 1.25% (T_2)
3. Rifampicin @ 2.75%, 3.0% and 3.25% (T_3)

3.1.3 Treatment details

No. of treatments: 03 + 03

Control I: untreated

Control II: Distilled water treated

Control III: Untreated healthy

No. of replications per treatment: 03

No. of worms per replication/treatment: 200

Design of the experiment: CRD (Completely Randomized Block Design).

3.2 Methods of study

Moths of the pebrinised seeds were thoroughly examined under microscope individually for the presence of pebrine infection. The seeds of those moths which exhibited 100 percent spore load were involved in the study. The diseased seeds were kept under hibernation as per the standard procedure (Kamili ad Masoodi, 2000 ad Rajan *et al.*, 2005) and were released from the cold storage for incubation on 13-04-18 .During the incubation a portion of seeds of infected layings were examined microscopically to ensure that the seeds under incubation are having full spore load of pebrine (*Nosema*). The rearing of pebrinised silkworms was carried out by following the standard methods of rearing (Krishnaswamy *et al.*, 1973). After hatching and brushing, the samples of first age and second age larvae were again examined microscopically to confirm and ensure further that the first age silkworms under rearing are infected with pebrine infection.

3.3 Effect of chemicals on the management of pebrine disease of silkworms

Three locally available low cost chemicals viz., sodium bicarbonate, ornidazole and rifampicin were evaluated under study to record their efficacy against the pebrine caused by protozoan parasite, (*Nosema* spp). The rearing and management study was conducted *in vivo*. The pebrinised worms were reared in mass upto 2^{nd} age. The 3^{rd} age larvae were counted and divided into three replications with 200 worms per replicate.

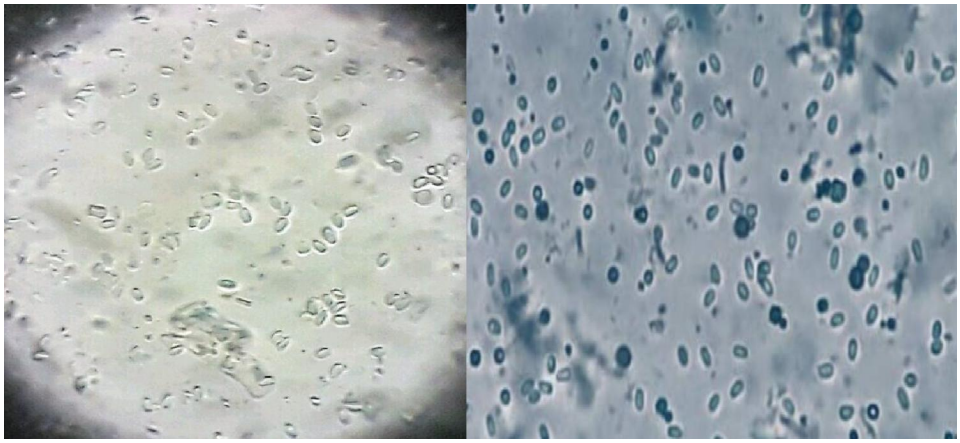
Of the three chemicals viz., sodium bicarbonate, ornidazole and rifampicin involved in the study, the ornidazole and rifampicin were in tablet form whereas sodium bicarbonate was in powdered form. The tablets of ornidazole and rifampicin were crushed to make the powder of these chemicals. The concentration of three different chemicals under study were prepared by dissolving the given chemicals in 1 litre of distilled water. On the basis of active

Silkworm rearing of 5th age larvae

Silkworm rearing at research site

Plate-1: Silkworm rearing

Larval examination under microscope



Pebrine spores under microscope

Plate-2: Microscopic examination at seed, larval and moth stage

ingredient of chemicals the required concentrations were obtained. The sodium bicarbonate was used at 0.6, 0.7 and 0.8%. concentrations, and prepared by dissolving 6 grams, 7 grams and 8 grams of sodium bicarbonate separately in 1 litre of distilled water. The ornidazole was used at 0.75, 1.0 and 1.25%. The said concentrations of ornidazole were prepared by dissolving ornidazole tablets 9.5, 11.5 and 13.5 separately in 1 litre of distilled water. The rifampicin was used at 2.75, 3.0 and 3.25%. The three concentrations of rifampicin were obtained by dissolving 5, 6 and 7 tablets separately in 1 litre of distilled water.

The different concentrations of the chemicals under study were sprayed on the mulberry leaves which was fed to silkworms of all the treatments once during the 3^{rd} age. During the 4^{th} age the treated leaves were given twice to silkworms under study while as during the 5^{th} age the treatment was given on the alternate days to check the efficacy of these chemicals towards pebrine infected larvae. For each treatment a separate untreated batch of the worms was kept as standard check Control-I, another batch of worms treated with distilled water was kept as control-II. In order to study the effect of chemicals under study on the commercial characters of the treated SKUAR-6 under rearing with the GP Bank of the College was used for comparison.

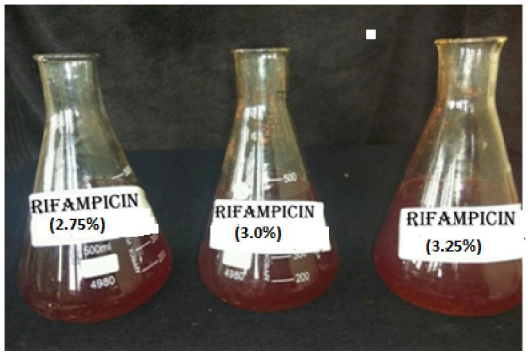
Following observations were recorded during the investigation.

3.3.1 Percent larval survival

The survival percentage of 3^{rd} age larvae was recorded and the survival percentage of 4^{th} and 5^{th} age larvae was also recorded alternately by the following formula:

$$\text{Larval survival (\%)} = \frac{\text{Larvae obtained at the end of } 3^{rd} \text{ age}}{\text{Total No. of larvae retained after } 2^{nd} \text{ moult}} \times 100$$

$$\text{Larval survival (\%)} = \frac{\text{Larvae obtained at the end of } 4^{th} \text{ age}}{\text{Total No. of larvae retained after } 2^{nd} \text{ moult}} \times 100$$



Different concentrations of chemicals under study

Application of chemicals to the silkworms under study

Plate-3: Preparation and application of chemicals on mulberry leaves

$$\text{Larval survival (\%)} = \frac{\text{Larvae obtained at the end of } 5^{th} \text{ age}}{\text{Total No. of larvae retained after } 2^{nd} \text{ moult}} \times 100$$

3.3.2 Effective rate of rearing (by number):

It was determined by the following formula:

$$\text{Rate of rearing (by No.)} = \frac{\text{No. of cocoons harvested}}{\text{No. of worms retained after } 3^{rd} \text{ moult}} \times 10{,}000$$

3.3.3 Effective rate of rearing (by weight):

It was determined by the following formula:

$$\text{Rate of rearing (by weight)} = \frac{\text{No. of cocoons harvested (kg)}}{\text{No. of worms retained after } 3^{rd} \text{ moult}} \times 10{,}000$$

3.3.4 Percentage of live cocoons

It was calculated on the basis of number of live pupae obtained from the cocoons harvested from each treatment & replicate by the following formula:

$$\text{Live cocoon (\%)} = \frac{\text{No. of live pupae obtained}}{\text{Total No. of cocoons harvested}} \times 100$$

3.3.5 Moth emergence percentage

Moth emergence for each replicate and treatment was recorded and calculated by the following formula:

$$\text{Moth emergence (\%)} = \frac{\text{No. of live moths emerged}}{\text{Total No. of cocoons per replicate/treatment}} \times 100$$

3.3.6 Percentage of average disease free layings obtained

Moths obtained were allowed to lay eggs and after that subjected to microscopic examination to ascertain the recovery of disease free moths and seeds. This was calculated by the following formula:

$$\text{Disease free layings (\%)} = \frac{\text{No. of disease free months per treatment/replication}}{\text{Total No. of moths examined per treatment/replication}} \times 100$$

3.4 Effect of chemicals on the commercial cocoon characters of silkworms

3.4.1 5^{th} instar larval duration (days)

It was recorded as number of days taken by 5^{th} age silkworms from day one of 5^{th} age till the seriposition.

3.4.2 Average weight of ten matured larvae (g)

Ten matured larvae were randomly picked from each treatment & replication one day before spinning and weighed to determine the average mature larval weight.

3.4.3 Total larval duration (days/hours)

It was recorded as total time taken by the silkworms under study from date of brushing of silkworms to mounting**.**

3.4.4 Average single cocoon weight (g)

10 male and 10 female cocoons were randomly selected from each replicate and treatment and weighed to determine average single cocoon weight.

3.4.5 Average single shell weight (g)

The cocoons used for determining average single cocoon weight were cut open to obtain the shells .The resultant shells were weighed to determine the average shell weight.

3.4.6 Shell ratio percentage

It was determined by the following formula:

$$\text{Shell ratio} = \frac{\text{Single shell weight}}{\text{Single cocoon weight}} \times 100$$

3.4.7 Average cocoon filament length (m)

Ten randomly selected cocoons of each treatment/replicate were stifled and reeled individually to record the total filament length of the cocoon obtained from each treatment/replicate.

Chapter 4

EXPERIMENTAL FINDINGS

The data pertaining to larval survival percentage, effective rate of rearing (by number and weight), percentage of live cocoons, moth emergence percentage, recovery of average Disease free layings (dfl) percentage, 5th instar larval duration, weight of 10 matured larvae, total larval duration, single cocoon weight, single shell weight,shell ratio and cocoon filament length was subjected to statistical analysis. The results obtained are presented in tables (1-15):

4.1 Larval survival percentage in 3rd age

Among the three chemicals viz., Sodium bicarbonate, Ornidazole, Rifampicin under study, the maximum average mean larval survival percentage (92.62) in 3rd age silkworms was shown by Sodium bicarbonate and minimum (87.06%) was shown by Rifampicin which was however statistically at par with the treatment Ornidazole (87.96%).

Among the different chemical concentrations under study, Sodium bicarbonate at 0.8 per cent concentration recorded the significantly highest survival percentage of 93.17 per cent, followed by 93.13 per cent (at 0.7% concentration) and 91.57 per cent (at 0.6 per cent concentration); Ornidazole at 0.75 per cent concentration exhibited the lowest value (86.89%). Ornidazole at 1.0 and 1.25 per cent concentration recorded the larval survival percentage of 87.32 and 89.67 per cent, respectively. Among the three concentrations of Rifampicin 3.25 per cent concentration recorded the highest larval survival percentage (88.33) followed by 3.0 per cent concentration and 2.75 per cent concentration recorded a larval survival percentage of 86.98 and 85.89 per cent, respectively.

Table 1 shows that all the three chemicals viz., under study have shown significantly higher larval survival percentage in 3rd age over control and with the increase in the concentrations, all these chemicals have shown increased response in their effectiveness.

Table 1: Effect of various chemicals on larval survival (%) in 3rd age

Treatments	Concentrations (%)	Survival (%)	Mean
Sodium bicarbonate	0.6	91.57	**92.62**
	0.7	93.13	
	0.8	93.17	
Ornidazole	0.75	86.89	**87.96**
	1.0	87.32	
	1.25	89.67	
Rifampicin	2.75	85.89	**87.06**
	3.0	86.98	
	3.25	88.33	
Control treatments	T_{00}(Untreated) : 64.19 T_{01} (Distilled water) : 63.59		

C.D ($p \leq 0.05$)

Treatment : 1.02

T×C : 1.12

Control vs Rest : 1.01

4.2 Larval survival percentage in 4th age

Table 2 indicates that among the chemicals viz., Sodium bicarbonate, Ornidazole and Rifampicin the maximum average mean larval survival percentage (87.94) was shown by Sodium bicarbonate and minimum (82.43) by Rifampicin. However, Ornidazole recorded (82.57%) average mean larval survival but the results of Ornidazole and Rifampicin were statistically at par with each other.

The highest (89.22%) larval survival percentage among the different concentrations was shown by sodium bicarbonate (at 0.8%) and lowest by Ornidazole (81.32) at 0.75 per cent concentration. In the treatment Sodium bicarbonate the highest larval survival (89.22%) was recorded at 0.8 per cent concentration and lowest (86.37%) at 0.6 per cent concentration. The Ornidazole exhibited the highest larval survival (84.33%) at 1.25 per cent concentration and lowest (81.32%) at 0.75 per cent concentration. Whereas, the Rifampicin showed the the highest larval survival (83.55%) at 3.25 per concentration and the lowest (81.62%) at 2.75 per cent concentration.

The results have revealed that all the three treatments have shown significant improved larval survival percentage over control (untreated). The overall results have shown increase in the effect of chemicals with the increase in the concentration.

4.3 Larval survival percentage in 5th age

It is revealed from the results of Table 3 that among the chemicals evaluated in the study programme, the results revealed that maximum average mean larval survival percentage (85.90) was shown by Sodium bicarbonate and minimum (78.87) by Rifampicin. Ornidazole however, exhibited average mean larval survival percentage (79.24%) but, the results of treatment Ornidazole were statistically at par with Rifampicin.

Table 2: Effect of various chemicals on larval survival (%) in 4th age

Treatments	Concentrations (%)	Survival (%)	Mean
Sodium bicarbonate	0.6	86.37	**87.94**
	0.7	88.23	
	0.8	89.22	
Ornidazole	0.75	81.32	**82.57**
	1.0	82.06	
	1.25	84.33	
Rifampicin	2.75	81.62	**82.43**
	3.0	82.13	
	3.25	83.55	
Control treatments	T_{00}(Untreated) : 38.11 T_{01} (Distilled water) : 37.40		

C.D ($p \leq 0.05$)

Treatment	:	0.48
T×C	:	0.31
Control vs Rest	:	1.08

Among the different concentrations under the study the treatment sodium bicarbonate at 0.8 per cent concentration exhibited highest larval survival (87.39%) and minimum (77.32%) by Ornidazole at 0.75 per cent concentration. Sodium bicarbonate recorded the highest larval survival (87.39%) at 0.8 per cent concentration and lowest (84.27%) at 0.6 per cent concentration. In the treatment Ornidazole, the highest larval survival (81.19%) was shown at 1.25 per cent concentration and lowest (77.32%) at 0.75 per cent concentration. However, Rifampicin recorded the maximum larval survival (80.07%) at 3.25 per cent concentration and the minimum (78.11%) at 2.75 per concentration.

The results of the Table 3 shows that the effect of all the three chemicals under study on the larval survival was significantly higher than control (untreated). The overall results have shown increase in the effect of chemicals with the increase in the concentration.

4.4 5^{th} instar larval duration (h)

The results of Table 4 revealed non-significant impact of all three treatments on the 5^{th} instar larval duration. Sodium bicarbonate recorded average mean 5^{th} instar larval duration (165 h), followed by Ornidazole and Rifampicin with 166 h. Among the concentrations under study the maximum 5^{th} instar larval duration (167 h) was recorded at 0.75, 2.75 and 3.0 per cent concentration of Ornidazole and Rifampicin, respectively. The minimum 5^{th} instat larval duration (164 h) at 0.8 per cent concentration was recorded by Sodium bicarbonate. However, statistically no significant difference was recorded among the treatments under study and the control batch (untreated healthy).

4.5 Weight of ten matured larvae (g)

The results did not reveal any significant difference among the three treatments under study and the control batch (untreated healthy). However, the highest average mean weight of larvae 50.61 g among the treatments under study was recorded by sodium bicarbonate followed by Ornidazole (50.47 g) and

Table 3: Effect of various chemicals on larval survival (%) in 5th age

Treatments	Concentrations (%)	Survival (%)	Mean
Sodium bicarbonate	0.6	84.27	**85.90**
	0.7	86.17	
	0.8	87.39	
Ornidazole	0.75	77.32	**79.24**
	1.0	79.21	
	1.25	81.19	
Rifampicin	2.75	78.11	**78.87**
	3.0	78.42	
	3.25	80.07	
Control treatments	**T_{00}(Untreated) : 11.39** **T_{01} (Distilled water) : 11.18**		

C.D ($p \leq 0.05$)

Treatment : 0.91

T×C : 0.18

Control vs Rest : 0.85

Table 4: Effect of various chemicals on 5th instar larval duration

Treatments	Concentrations (%)	5th instar larval duration (h)	Mean (h)
Sodium bicarbonate	0.6	165	**165**
	0.7	165	
	0.8	164	
Ornidazole	0.75	167	**166**
	1.0	166	
	1.25	166	
Rifampicin	2.75	167	**166**
	3.0	167	
	3.25	165	
Control treatments	Untreated healthy : 165		

C.D ($p \leq 0.05$)

Treatment : NS

TXC : NS

Control vs Rest : NS

Rifampicin (50.13 g). The treatments Ornidazole and Rifampicin were statistically at par.

Among the concentrations the highest larval weight (51.80 g) was shown at 1.25 per cent concentration of Ornidazole and lowest (49.33 g) was revealed at 0.75 and 2.75 per cent concentration of Ornidazole and Rifampicin, respectively. In the treatment Sodium bicarbonate the highest larval weight (51.46 g) was recorded at 0.8 per cent concentration and lowest (50.16 g) at 0.6 per cent concentration. Ornidazole recorded the highest larval weight (51.80 g) at 1.25 per cent concentration and lowest (49.33 g) at 0.75 per cent concentration. However, treatment Rifampicin recorded the highest larval weight (50.93 g) at 3.25 per cent concentration and the lowest (49.33 g) at 2.75 per cent concentration (Table 5).

4.6 Total larval duration (h)

It is evident from the results presented in Table-6 that there is no significant difference in the total larval duration among the three treatments under study. Among the three treatments under study, Sodium bicarbonate recorded total larval duration of (608 h) followed by Rifampicin (609 h) and Ornidazole (611 h). The maximum total larval duration (611 h) among the concentrations under study was recorded by Sodium bicarbonate at 0.6 per cent concentration and minimum (604 h) at 0.8 per cent concentration. In the treatment Ornidazole the highest total larval duration 614 h was observed at 0.75 per cent concentration and lowest (609 h) at 1.25 per cent concentration. Whereas, Rifampicin recorded the highest value 612 h of total larval duration at 2.75 per cent concentration and minimum (605 h) at 3.25 per cent concentration. (Table 6).

4.7 Cocoon yield by number

Table 7 reveals the maximum average mean cocoon yield by number (8350) was recorded by Sodium bicarbonate and minimum (7700) by the treatment Rifampicin. Ornidazole recorded 7683 average mean cocoon yield by number. The results revealed that with increase in the concentration of chemicals

Table 5: Effect of various chemicals on weight of 10 matured larvae

Treatments	Concentrations (%)	Weight of 10 matured larvae (g)	Mean (g)
Sodium bicarbonate	0.6	50.16	**50.61**
	0.7	50.20	
	0.8	51.46	
Ornidazole	0.75	49.33	**50.47**
	1.0	50.27	
	1.25	51.80	
Rifampicin	2.75	49.33	**50.13**
	3.0	50.13	
	3.25	50.93	
Control treatments	Untreated healthy : 50.57		

C.D ($p \leq 0.05$)

Treatment : NS

T×C : NS

Control vs Rest : 2.01

Table 6: Effect of various chemicals on total larval duration

Treatments	Concentrations (%)	Total larval duration (h)	Mean (g)
Sodium bicarbonate	0.6	611	**608**
	0.7	609	
	0.8	604	
Ornidazole	0.75	614	**611**
	1.0	611	
	1.25	609	
Rifampicin	2.75	612	**609**
	3.0	610	
	3.25	605	
Control treatments	Untreated healthy : 604		

C.D ($p \leq 0.05$)

Treatment : NS

T×C : NS

Control vs Rest : NS

the cocoon yield by number increased: Sodium bicarbonate at 0.6, 0.7 and 0.8 per cent concentrations exhibited cocoon yield (by number) of 8100, 8400 and 8550 respectively. Ornidazole recorded a value of 7400, 7700 and 7950 of cocoon yield (by number) at 0.75, 1.0 and 1.25 per concentrations, respectively. Similarly, Rifampicin recorded a value of 7550, 7700 and 7850 of cocoon yield (by number). All the three treatments under study showed improved cocoon yield by number over control (untreated).

4.8 Cocoon yield by weight (kg)

The treatments under study revealed maximum average mean cocoon yield (by weight) 19.28 kg by treatment Sodium bicarbonate. Ornidazole recorded 17.56 kg cocoon yield by weight followed by Rifampicin (17.55 kg). However, both Ornidazole and Rfampicin were statistically at par.

Among the different concentrations under study the highest cocoon yield by weight (19.92 kg) was recorded by Sodium bicarbonate at 0.8 per cent concentration and lowest (16.79 kg) at 0.75 per cent concentration of Ornidazole. Sodium bicarbonate exhibited the highest cocoon yield by weight (19.92 kg) at 0.8 per cent concentration and lowest (18.54 kg) at 0.6 per cent concentration. In the treatment Ornidazole the highest cocoon yield by weight (18.36 kg) was shown at 1.25 per cent concentration and lowest (16.79 kg) at 0.75 per cent concentration. However, Rifampicin recorded the highest cocoon yield by weight (18.05 kg) at 3.25 per cent concentration and the lowest (17.13 kg) at 2.75 per concentration.

Table 8 reveals that all the three treatments under study have shown significant improvement over the control (untreated). The overall results have shown increase in the effect of chemicals with the increase in the concentration (Table 8).

Table 7: Effect of various chemicals on cocoon yield by number

Treatments	Concentrations (%)	Cocoon yield by number	Mean (g)
Sodium bicarbonate	0.6	8100	**8350**
	0.7	8400	
	0.8	8550	
Ornidazole	0.75	7400	**7683**
	1.0	7700	
	1.25	7950	
Rifampicin	2.75	7550	**7700**
	3.0	7700	
	3.25	7850	
Control treatments	T_{00}(Untreated) : 0.00 T_{01} (Distilled water) : 0.00		

C.D ($p \leq 0.05$)

Treatment	:	59.49
T×C	:	110
Control vs Rest	:	116.95

Table 8: Effect of various chemicals on cocoon yield by weight

Treatments	Concentrations (%)	Cocoon yield by weight (kg)	Mean (kg)
Sodium bicarbonate	0.6	18.54	**19.28**
	0.7	19.40	
	0.8	19.92	
Ornidazole	0.75	16.79	**17.56**
	1.0	17.55	
	1.25	18.36	
Rifampicin	2.75	17.13	**17.55**
	3.0	17.47	
	3.25	18.05	
Control treatments	T_{00}(Untreated) : 0.00 T_{01} (Distilled water) : 0.00		

C.D ($p \leq 0.05$)

Treatment : 0.81

T×C : 0.61

Control vs Rest : 1.44

4.9 Percentage of live cocoons

Table 9 reveals the 95.84 maximum average mean percentage of live cocoons recorded by Sodium bicarbonate and the minimum (92.94) by the treatment Rifampicin. Whereas, the treatment Ornidazole recorded the average mean value of 93.86 per cent. Among the different concentrations under study the highest (97.80) live cocoon percentage was recorded at 0.8 per cent concentration of Sodium bicarbonate and the lowest (90.87) at 2.75 per cent concentration of Rifampicin. Sodium bicarbonate recorded the highest 97.80 per cent live cocoons at 0.8 per cent concentration and lowest 94.44 per cent live cocoons at 0.6 per cent concentration. The treatment Ornidazole recorded the maximum live cocoon percentage (95.74) at 1.25 per cent concentration and lowest (91.32) at 0.75 per cent concentration. In the treatment Rifampicin the highest live cocoon percentage (94.56) was recorded at 3.25 per cent concentration and the lowest (90.87) was shown at 2.75 per concentration.

All the three treatments under study have shown significant improvement over control (untreated). The overall results have shown increase in the effect of chemicals with the increase in the concentration.

4.10 Average single cocoon weight

Among the three chemicals under study, the average mean single cocoon weight (2.34 g) was recorded by treatment Sodium bicarbonate followed by Ornidazole (2.33 g) and Rifampicin (2.31 g). Among the different concentrations under study, the highest single cocoon weight (2.39 g) was observed in the treatment Sodium bicarbonate at 0.8 per cent concentration and the lowest (2.28 g) was shown at 0.75 concentration of Ornidazole. Sodium bicarbonate at 0.8 per cent concentration recorded the highest (2.39 g) single cocoon weight and lowest single cocoon weight (2.32 g) at 0.6 per cent concentration. The treatment Ornidazole revealed the highest single cocoon weight (2.37 g) at 1.25 per cent concentration and lowest (2.28 g) at 0.75 per cent concentration. Rifampicin

Table 9: Effect of various chemicals on percentage of live cocoons

Treatments	Concentrations (%)	Percentage of live cocoons	Mean (g)
Sodium bicarbonate	0.6	94.44	**95.84**
	0.7	95.28	
	0.8	97.80	
Ornidazole	0.75	91.32	**93.86**
	1.0	94.53	
	1.25	95.74	
Rifampicin	2.75	90.87	**92.94**
	3.0	93.40	
	3.25	94.56	
Control treatments	T_{00}(Untreated) : 0.00 T_{01} (Distilled water) : 0.00		

C.D ($p \leq 0.05$)

Treatment : 0.96

T×C : 0.39

Control vs Rest : 1.01

Table 10: Effect of various chemicals on average single cocoon weight

Treatments	Concentrations (%)	Average single cocoon weight (g)	Mean (g)
Sodium bicarbonate	0.6	2.32	**2.34**
	0.7	2.33	
	0.8	2.39	
Ornidazole	0.75	2.28	**2.33**
	1.0	2.33	
	1.25	2.37	
Rifampicin	2.75	2.34	**2.31**
	3.0	2.35	
	3.25	2.36	
Control treatments	Untreated healthy : 2.34		

C.D ($p \leq 0.05$)

Treatment	:	0.02
TXC	:	0.03
Control vs Rest	:	1.56

recorded the maximum single cocoon weight (2.36 g) at 3.25 per cent concentration and the minimum (2.34 g) at 2.75 per concentration. However, all the three treatments were found at par with control batch (untreated healthy) (Table 10).

4.11 Average single shell weight (cg)

The maximum average mean single shell weight (0.40 cg) was recorded by the treatment Sodium bicarbonate. The treatments Ornidazole and Rifampicin recorded 0.36 cg average mean single shell weight. However, the two treatments Ornidazole and Rifampicin were statistically at par. Among the different concentrations under study, the highest single shell weight (0.43 cg) was observed at 0.8 per cent concentration of Sodium bicarbonate and the lowest (0.35 cg) was recorded at 0.75, 2.75 and 3.0 per cent concentration of treatments Ornidazole and Rifampicin, respectively. In the treatment Sodium bicarbonate the highest single shell weight (0.43 cg) was shown at 0.8 per cent concentration and lowest (0.38 cg) at 0.6 per cent concentration. Ornidazole revealed the highest single shell weight (0.39 cg) at 1.25 per cent concentration and lowest (0.35cg) at 0.75 per cent concentration. However, the treatment Rifampicin recorded maximum single cocoon weight of 0.38 cg at 3.25 per cent concentration and minimum (0.35 cg) at 2.75 and 3.0 per cent concentrations. All the three treatments were found at par with control batch (untreated healthy). The overall results have shown increase in the effect of chemicals with the increase in the concentration (Table 11).

4.12 Shell percentage

The results presented in Table 12 reveal that sodium bicarbonate recorded average mean shell percentage (18.74) per cent followed by Ornidazole (18.47) and Rifampicin (18.40). The results revealed non-significant impact of all the three treatments under study on shell percentage and the control batch (untreated healthy). Among the different concentrations the highest shell percentage (19.66)

Table 11: Effect of various chemicals on average single shell weight

Treatments	Concentrations (%)	Average single shell weight (cg)	Mean (cg)
Sodium bicarbonate	0.6	0.38	**0.40**
	0.7	0.39	
	0.8	0.43	
Ornidazole	0.75	0.35	**0.36**
	1.0	0.36	
	1.25	0.39	
Rifampicin	2.75	0.35	**0.36**
	3.0	0.35	
	3.25	0.38	
Control treatments	Untreated healthy : 0.42		

C.D ($p \leq 0.05$)

Treatment : 0.02

T×C : 0.03

Control vs Rest : 0.09

Table 12: Effect of various chemicals on shell percentage

Treatments	Concentrations (%)	Shell percentage	Mean
Sodium bicarbonate	0.6	18.10	**18.74**
	0.7	18.45	
	0.8	19.66	
Ornidazole	0.75	17.98	**18.47**
	1.0	18.03	
	1.25	19.41	
Rifampicin	2.75	17.69	**18.40**
	3.0	18.37	
	3.25	19.14	
Control treatments	Untreated healthy : 18.70		

C.D ($p \leq 0.05$)

Treatment : NS

T×C : NS

Control vs Rest : 1.55

was recorded in the treatment sodium bicarbonate at 0.8 per cent concentration and lowest (17.69) at 2.75 per cent concentration in the treatment Rifampicin.

Among the different treatments under study, Sodium bicarbonate recorded the highest shell percentage (19.66) at 0.8 per cent concentration and lowest (18.10) at 0.6 per cent concentration. However, the treatment Ornidazole revealed the highest shell percentage (19.41) at 1.25 per cent concentration and lowest (17.98) at 0.75 per cent concentration. The treatment Rifampicin has shown the maximum shell percentage (19.14) at 3.25 per cent concentration and the lowest (17.69) at 2.75 per concentration.

4.13 Moth emergence percentage

Among the three treatments evaluated in the study programme, the maximum average mean moth emergence percentage (94.71) was recorded in the treatment Sodium bicarbonate and the minimum (92.36) in the treatment Rifampicin. Ornidazole however, recorded 92.48 per cent moth emergence.

Among the different concentrations under study, the highest moth emergence percentage (95.93) at 0.8 per cent concentration was shown by the treatment Sodium bicarbonate and the lowest (91.43) at 2.75 per cent concentration of treatment Rifampicin. In the treatment Sodium bicarbonate the highest moth emergence percentage (95.93) was shown at 0.8 per cent concentration and lowest (93.61) at 0.6 per cent concentration. Ornidazole recorded the maximum moth emergence percentage (94.12) at 1.25 per cent concentration and lowest (91.52) at 0.75 per cent concentration. In the treatment Rifampicin the highest moth emergence percentage (93.53) was recorded at 3.25 per cent concentration and the lowest (91.43) at 2.75 per concentration.

All the three treatments under study have shown significant improvement over control (untreated). The overall results have shown increase in the effect of chemicals with the increase in the concentration. (Table 13).

Table 13: Effect of various chemicals on moth emergence percentage

Treatments	Concentrations (%)	Moth emergence percentage	Mean
Sodium bicarbonate	0.6	93.61	**94.71**
	0.7	94.58	
	0.8	95.93	
Ornidazole	0.75	91.52	**92.48**
	1.0	91.81	
	1.25	94.12	
Rifampicin	2.75	91.43	**92.36**
	3.0	92.12	
	3.25	93.53	
Control treatments	T_{00}(Untreated) : 0.00 T_{01} (Distilled water) : 0.00		

C.D ($p \leq 0.05$)

Treatment : 0.52

T×C : 0.31

Control vs Rest : 1.19

4.14 Recovery percentage of DFLs

The results of Table 14 reveal that the maximum average mean recovery percentage of dfl's (95.72) among the treatments under study was recorded in the treatment Sodium bicarbonate and the minimum (93.91) by Rifampicin. The recovery percentage of dfl's in the treatment Ornidazole was observed (94.07%). Among the different concentrations under study, the highest recovery percentage of dfl's (96.31) at 0.8 per cent concentration was recorde by Sodium bicarbonate and the lowest (92.93) at 3.0 per cent concentration by treatment Rifampicin.

Among the different concentrations under study, the treatment Sodium bicarbonate recorded the highest recovery percentage of dfl's (96.31) at 0.8 per cent concentration and lowest (95.20) at 0.6 per cent concentration. The treatment Ornidazole showed the maximum recovery percentage of dfl's (95.40) at 1.25 per cent concentration and lowest (93.29) at 0.75 per cent concentration. In the treatment Rifampicin the highest recovery percentage of dfl's (95.55) was recorded at 3.25 per cent concentration and the lowest (93.25) at 2.75 per concentration. All the three treatments under study have shown significant improvement over control (untreated). The overall results have shown increase in the effect of chemicals with the increase in the concentration (Table 14).

4.15 Average silk cocoon filament length (m)

The Table 15 revealed that among the treatments the average mean silk filament length in Sodium bicarbonate (1190 m) was recorded followed by Rifampicin (1166 m) and Ornidazole (1160 m).However, no significant difference was revealed among the three treatments and control batch (untreated healthy). Among the different concentrations, the highest silk filament length (1205 m) was shown at 0.8 per cent concentration of Sodium bicarbonate and the lowest (1127 m) at 0.75 per cent concentration by Ornidazole. In the treatment Sodium bicarbonate the highest silk filament length (1205 m) was observed at 0.8 per cent concentration and lowest (1179) at 0.6 per cent concentration. However, in

Ornidazole the maximum silk filament length (1197 m) was recorded at 3.25 per cent concentration and lowest (1144 m) at 3.0 per cent concentration. The treatment Rifampicin recorded the highest (1198 m) silk filament length at 3.25 per cent concentration and lowest (1156 m) at 2.75 per cent concentration. The overall results have shown increase in the effect of chemicals with the increase in the concentration.

Table 14: Effect of various chemicals on recovery percentage of DFLs

Treatments	Concentrations (%)	Recovery percentage of DFLs	Mean
Sodium bicarbonate	0.6	95.20	**95.72**
	0.7	95.66	
	0.8	96.31	
Ornidazole	0.75	93.29	**94.07**
	1.0	93.52	
	1.25	95.40	
Rifampicin	2.75	93.25	**93.91**
	3.0	92.93	
	3.25	95.55	
Control treatments	T_{00}(Untreated) : 0.00 T_{01} (Distilled water) : 0.00		

C.D ($p \leq 0.05$)

Treatment	:	0.89
T×C	:	1.55
Control vs Rest	:	1.39

Table 15: Effect of various chemicals on length of cocoon filament

Treatments	Concentrations (%)	Length of silk filament (m)	Mean (m)
Sodium bicarbonate	0.6	1179	**1190**
	0.7	1188	
	0.8	1205	
Ornidazole	0.75	1127	**1160**
	1.0	1157	
	1.25	1197	
Rifampicin	2.75	1156	**1166**
	3.0	1144	
	3.25	1198	
Control treatments	Untreated healthy: 1194		

C.D ($p \leq 0.05$)

Treatment : NS

T×C : 32.25

Control vs Rest : 170.41

Cocoons harvested during study

Reeling of cocoons harvested during the research trial

Plate-4: Reeling of harvested cocoons

Chapter 5

DISCUSSION

The mulberry silkworm (*Bombyx mori* L.) has been under domestication for over 5000 years now and has been utilized by man for its needs and variety of benefits. Prolonged domestication and continuous rearing has exposed this beneficial insect to various pathogenic micro-organisms. The most dangerous out of these is a parasite attacking the silkworm which belongs to a group of primitive obligate eukaryotic intracellular parasite i.e., Microsporidia and genus *Nosema*. This microsporidian is highly dangerous and can wipe out the silk industry if not addressed. Earlier, also it has wiped out the silk industry in some of the European countries (Frace and Itlay) as early as in 1845. Many Nosema sp. N. bombycis, Pleistophora sp, NIK-2r, NIK-4m, NIK-5hm, Nosema sp., NIAP-6p, NIK-5d, NIK-1Pr, NIK-1Cc, NIK-1Cpy, NIK-1So, NIK-1Dp. have been reported to attack the silkworms (Bhat and Kamili,2009) causing pebrine disease of different magnitudes. Several microsporidia other than *Nosema bombycis* like *Nosema apis, Varimorpha* sp*., Pleistophora* sp*., Thelohania* sp. etc. are also reported to cause pebrine disease in different insects (Chandrasekharan *et al.,* 2017). Microsporidia have even been reported to infect honey bees, fish etc. *Glugea* sp. attacks fish while as several *Nosema* sp. infect and destroy silkworms and also honey bee colonies. Microsporidia even infect almost all animal phyla and many of them have been reported as causative agents of some human diseases (Chandrasekharan *et al.*, 2017). All the microsporidian species mainly transmit the disease by ingestion of spores (horizontal transmission). In addition, many spores of microsporidia such as *Nosema* sp. attacking silkworms are transmitted vertically or transovarially from an infected adult female to the offspring. Pebrine disease in the silkworms has been reported to infect all the stages and breeds of the silkworms through primary and secondary contamination posing a great threat to the industry.

The dreadful disease 'Pebrine' has been responsible for ruining the silk industry in France and Itlay (Kamili and Masoodi, 2000). Records reveal that the disastrous effect of the pebrine disease was even felt in Kashmir as the productive indigenous univoltine breed "Kashmir Race" was lost due to outbreak of pebrine disease some 131 years back (Kamili and Masoodi, 2000). Extensive researches have brought into effect some of the ways for the management of this dreadful pebrine disease but this microsporidian infection still remains a major threat to the sericulture industry with its recurrent occurence and transovarial transmission. The earliest research on pebrine was confined to the epizootology and prevention (Weiser, 1969; Ishihara, 1963; Fujiwara, 1979). The management of the pebrine disease later became a key factor in preventing the cocoon loss to the farmers as the disease has immense potential to wipe out the whole crop.

Although many chemicals have been earlier tried for the management of pebrine disease in silkworms (Jolly *et al.*, 1981; Chandra & Kund, 1982-83; Liu Shi Xian, 1987; Griyaghey *et al.*, 1987) but the results did not show any significant control over the disease. Keeping in view the disastrous consequences of the pebrine disease and less availability of management practices which would provide significant control of the disease, the present study titled "Management of the Pebrine Disease in Bivoltine Silkworms Reared under Temperate Climatic Conditions" was taken in hand with a view to manage this dreadful disease and bring some relief to the silkworm rearers.

After scanning the literature, three chemicals v.i.z Sodium bicarbonate, Ornidazole and Rifampicin were taken for the study. These chemicals were selected in view of their efficacy in controlling the several protozoan diseases in many related insects and living creatures. The chemicals used for the study programme have also been reported to be effective against *Nosema apis* in honeybees, *Nosema* sp. infecting Encarsia wasp (a parasitic protozoa), *Nosema* sp. infecting Lamerin breeds of silkworm etc (Sheetz *et al.*, 1997 and Bhat *et al.*, 2012).

The sodium bicarbonate is highly recommended drug readily available in the pharmaceutical industries and also for controlling many agricultural pests and veterinary infections. 5.0% sodium bicarbonate has been used for the treatment of Babesia infection in mouse (Goo *et al.*, 2010), whereas, 0.7 per cent concentration of Sodium bicarbonate is used in controlling the protozoan parasite. *Nosema apis* attacking the honey bee colonies and has been found effective against the parasite. The Sodium bicarbonate has been used effectively for the treatment of metabolic acidiosis in humans (Adeva-Annday *et al.*, 2014).

In view of these results, Sodium bicarbonate at 0.6, 0.7 and 0.8 per cent concentrations was involved in the present study to see its effectiveness against the infection in Temperate silkworms caused by *Nosema* sp.

The chemical Ornidazole used in the present study has also been tested against protozoan diseases attacking the Lamerin breeds of silkworm (Bhat *et al.,* 2012). This chemical has also been seen to be used in the management of the Giardiasis (a protozoa parasite) in humans. Thus in the present study besides 1.0 per cent concentration of Ornidazole, 0.75 and 1.25 per cent concentration was also involved in the present study to identify the particular concentration with better efficacy.

The Rifampicin is a choice of drug for the protozoan infection in humans and has also been found effective at 3.0 per cent concentration against the Nosema disease in *Muscidufurax raptor* (an important bio-control agent against filth flies) (Sheetz *et al.*, 1997). In the present study besides used 3.0 per cent concentration of Rifampicin, two more concentrations like 2.75 and 3.25, were involved in the study to check the efficacy of the chemical.

5.1 Effect of chemicals on the management of pebrine disease

The results of the three chemicals viz., Sodium bicarbonate, Ornidazole and Rifampicin used in the present investigation for the management of pebrine disease are discussed for the following parameters.

5.1.1 Larval survival (%)

The larval survival is very important parameter and forms the base of good cocoon crop yield and good harvest as well for getting the healthy pupae, moths and seed for rearing next generation of silkworms. In the preset study the sodium bicarbonate, Ornidazole and Rifampicin were found effective for the management of pebrine disease as the larval survival percentage of the diseased worms exhibited a healthy trend. In general all the three chemicals used in the study have shown significantly good larval survival percentage after its administration in 3rd, 4th and 5th instar silkworms but Sodium bicarbonate at 0.8 per cent concentration gave the highest larval survival of 92.62 per cent, 87.94 per cent and 85.90 per cent in 3rd, 4th and 5th age silkworms, respectively. This was followed by Rifampicin showing 88.33 per cent, 83.55 per cent and 80.07% larval survival percentage in 3rd, 4th and 5th age, respectively. Ornidazole exhibited (85.89 per cent, 81.62 per cent and 78.11%) larval survival in 3rd, 4th and 5th age silkworms, respectively.

These findings are in conformity with the findings of (Bansal *et al.*, 1996) who reported 87 per cent survival in honey bees attacked by *Nosema apis* with the application of 0.7 per cent concentration of Sodium bicarbonate. Bhat *et al.* (2012) also reported 96.46 per cent reduction in larval mortality in silkworms by using Ornidazole at 1.0 per cent concentration.

A significant difference was recorded among the different treatments under study. As the concentration levels in all the three treatments increased, the larval survival also reflected the good efficacy of the chemicals under study.

5.1.2 Effective rate of rearing (by number and weight)

The cocoon yield by number and by weight is directly attributed to the larval weight since larval survival and larval weight are positively correlated with the yield (Effective Rate of Rearing). Among the three chemicals evaluated for their efficacy against the pebrine disease, the highest cocoon yield by number

(8550) and by weight (19.92 kg) was recorded at 0.8 per cent concentration of Sodium bicarbonate followed by Ornidazole (7950 yield by number and 18.36 kg yield by weight) at 1.25 per cent concentration .Rifampicin recorded 7850 number of cocoons and 18.05 kg cocoon yield at 3.25 per cent concentration. However, significant difference was observed among the treatments and the control (untreated).

The results are in conformity with the results recorded by Bhat *et al.* (2012) and Chandra *et al.* (1995) who have also reported significant improvement in Effective Rate of Rearing with the use of chemical Ornidazole at 1.0 per cent concentration and Sodium bicarbonate at 0.7 per cent concentration, respectively.

5.1.3 Percentage of live cocoons

Among the three chemicals the Sodium bicarbonate recorded the average highest percentage of live cocoons (95.84%) followed by Ornidazole (93.86%) and Rifampicin (92.94%). The maximum live cocoons (97.80%) was observed at 0.8 per cent concentration of treatment Sodium bicarbonate and minimum (90.87%) at 2.75 per cent concentration of treatment Rifampicin. Significant difference was exhibited among the treatments and the control (untreated).

The results revealed that management through the use of Sodium bicarbonate has helped the pupae of silkworms under study to metamorphose into adults. Highly infected pupae by pebrine disease have been reported to fail to metamorphose into adults (Singh *et al.*, 2003).This also confirms the high efficacy of Sodium bicarbonate in getting above 97 per cent live cocoons percentage.

5.1.4 Moth emergence percentage

Moth emergence is an important parameter as it is the moth laying the seed which helps in the continuation of life cycle of the silkworm (*Bombyx mori* L.). The percentage of emergence of mother moths is directly proportional to the egg layings which are preserved for continuation of following generations and cocoon crop harvest. Thus, when moth emergence percentage is higher, better egg

production is there which ultimately results in good crop of cocoons and better returns to the farmers.

Among the three chemicals used in the present study the highest average moth emergence (94.71%) was recorded in the treatment Sodium bicarbonate followed by Ornidazole (92.48%) and Rifampicin (92.36%). Among the different concentrations used, the highest moth emergence (95.93%) was shown by the treatment Sodium bicarbonate at 0.8 per cent concentration followed by Ornidazole (94.12%) at 1.25 per cent concentration and Rifampicin (94.56%) at3.25 per cent concentration. Statistically all the treatments revealed significant difference with each other

The emergence of moths free from pebrine is the most effective way to manage this dreadful disease since the pebrine spore is transmitted transovarially thus infecting the whole population (Han & Watanabe, 1988). Therefore, it is important to ensure that moths emerged are pebrine free. The present investigation is supported by the findings of Bhat *et al.* (2012) who have reported (59.34%) suppression of disease till moth stage with use of Sodium bicarbonate.

5.1.5 Recovery percentage of dfls

Obtaining the disease free layings is the ultimate goal for rearing the disease free silkworms population. If the seeds are not free from pebrine, it is obvious that embryo in the egg is loaded with the infection and it is sure that during the rearing transfer of infection to the next progeny will take place (Kamili and Masoodi, 2000). Thus recovery of disease free layings is the way for managing the dreadful pebrine disease and also to have a healthy cocoon crop. In the present study, the moth emergence percentage was highest in the treatment Sodium bicarbonate and the recovery percentage of dfl's (95.72%) was also highest in the Sodium bicarbonate. This was followed by Ornidazole (94.07%) and Rifampicin (93.91%). While recording the interaction of chemical and concentration, the maximum recovery percentage of dfl's (96.31%) was recorded

at 0.8 per cent concentration of Sodium bicarbonate followed by Rifampicin (95.55%) at 3.25 per cent concentration However, all the chemicals have shown significant difference with the control (untreated).

The present findings are in conformity with the results of (Ganie *et al.*, 2008) who reported that Ornidazole recorded (53.57%) fecundity at 0.4 per cent concentration. Sodium bicarbonate (0.8 per cent) have surpassed the results of Ganie *et al*., 2018 by getting 96.31 per cent of dfl recovery.

5.2 Effect of chemicals on the commercial cocoon characters of silkworms

The successful rearing of silkworms is associated with the larval and the cocoon characteristics such as larval duration, cocoon weight, shell weight, shell ratio and filament length etc. These parameters are directly influenced with the silkworm seed production, silk production as well as silk textiles. In other words, rearers, reelers, weavers and silk garment manufacturers are concerned with the performance of these parameters. Thus it was very important to study the effect of these chemicals under investigation on the following commercial cocoon parameters of silkworms.

5.2.1 Larval duration (5^{th} instar and total larval duration)

Duration of total life span effects the production cost of cocoons and silk reeling (Murakashi and Ohtsaki, 1989). The longer 5^{th} age and total larval duration of silkworms means consumption of more mulberry leaves, time ad money which is cost prohibitive. Therefore, rearers always desire that 5^{th} age duration of silkworms should not be more than (168- 192 hrs) in bivoltine temperate silkworm breeds and total larval duration should not be more than (648-672 hrs). Thus it was important to see the effect of chemicals under study on the 5^{th} age larval duration and total larval duration. In the present study out of three chemicals used Sodium bicarbonate recorded the 5^{th} instar larval duration (165 h) and total larval duration (608 h) followed by Rifampicin (166 h) 5^{th} instar larval duration and (609 h) total larval duration and longest total larval duration (611 h)

was recorded in the treatment Ornidazole. However, effect of all the treatments irrespective of their concentration were statistically at par. This clearly gives a indication that larval duration was not adversely affected by the use of the chemicals under study.

5.2.2 Weight of ten matured larvae

The maximum weight of fully grown silkworm larvae is the result of robust nature of a particular genotype. In bivoltine temperate breeds the weight of matured larvae of above 40 g is recommended. It has been also revealed that weight of silkworms has significant correlation with the yield contributing traits (Dar. 2014). In the present study maximum larval weight (50.61 g) was recorded in the treatment Sodium bicarbonate followed by Ornidazole (50.47 g) m and Rifampicin (50.13 g). The results obtained are statistically at par with control 50.57g (untreated healthy).

These findings are in conformity with those of Liu-Shi Xian (1987), Chandra *et al.* (1995) and Zargar *et al.* (2001) who have found that Topsin-M and Bavistin at 1.0 per cent concentration recorded 35.408 g and 35.208 g weight of ten matured larvae. However, the preset findings surpass the results of these authors and Sodium bicarbonate at 0.8 per cent exhibited the weight of ten matured larvae as high as 50.61 g reflecting the good efficacy of the chemical.

5.2.3 Single cocoon weight

Cocoon weight is an important economic parameter. The silkworm breeds having higher weight of cocoons are mostly preferred by silkworm rearers because such cocoons give more number of eggs as well as good silk content. Generally prices are paid to the forums on the basis of weight, as weight indicates the approximate quantity of raw silk that can be reeled from the cocoons (Krisnaswami *et al.*, 1981). In the present study, maximum average mean cocoon weight (2.34 g) was recorded in the lot treated by Sodium bicarbonate followed by Ornidazole (2.33 g) and Rifampicin (2.31 g). Significantly the highest cocoon

weight (2.39 g) was recorded at 0.8 per cent concentration of sodium bicarbonate followed by (2.37 g) at 1.25 per cent concentration of Ornidazole. Rifampicin gave the cocoon weight of 2.36 g which was at par with the control.

The effect of various chemicals upon the cocoon weight has been observed by Saha Kundu & Chandra (1979) and Jolly *et al.* (1981) who have found that the chemical Carbendazim resulted in increased single cocoon weight. The results are supported by the findings of Zargar *et al.* (2001) who has found 1.538 g of single cocoon weight with the use of chemical Metrogyl at 0.2 per cent concentration. However, the results of chemicals under study have surpassed the results of Zargar *et al*., (2001).

5.2.4 Single shell weight

Weight of cocoon shell has high significance, as it is the shell that yields the silk after reeling. Hence, higher the weight of the shell, higher the silk yield from it (Krisnaswami *et al.,* 1982). In the present study the highest shell weight (0.40 cg) was recorded in the treatment Sodium bicarbonate followed by Ornidazole and Rifampicin with (0.43 cg) shell weight. The maximum shell weight (0.43 cg) was observed in the treatment Sodium bicarbonate at 0.8 per cent concentration followed by Ornidazole (0.39 cg) at 1.25 per cent concentration. Results obtained from the treatments were found significantly at par with control (untreated healthy).

The present study is supported by the findings of Zargar *et al.* (2001) who has recorded 0.350 cg single shell weight with the use of chemical Codrinal at 0.1 per cent concentration. Similar findings were also made by Kundu and Chandra (1979). The findings of these authors however, gave a clue that the chemicals under study have give better results.

5.2.5 Shell percentage

It is an important commercial cocoon character as the shell ratio gives a fair indication of the quantity of raw silk that can be reeled from the lot of fresh

cocoons and therefore, helps in estimating the renditta which expresses the unit for production of 1 kg of silk (Krishnaswami *et al.*, 1981). Thus, it is the shell ratio which is generally valued in commercial transaction of cocoons. In the present study, the maximum average mean shell percentage (18.74%) was recorded in the treatment Sodium bicaronate followed by Ornidazole (18.47%) and rifampicin (18.40%). No, significant improvement over control was recorded among the treatments and the control (untreated healthy).

The present findings are in conformity with the Saha Kundu and Chandra, 1978 and Chandra *et al.* (1995). The findings of Zargar *et al.* (2001) also support the present study who has recorded (25.67) shell percentage with the use of chemical Codrinal at 0.1 concentration. However, higher shell percentage recorded by Zargar et al., (2001) could be attributed to the low cocoon weight and shell weight of results of these authors. I the present study both cocoon weight and shell weight were made higher which is directly related to the slightly lower cocoon shell percentage.

5.2.6 Average filament length (m)

Silk filament is the end result of rearing and reeling. The longer the filament length, the better it is for weavers and textile producers. Thus it was important to observe the effect of the chemicals under study on the cocoon filament length. The present investigation revealed that the maximum average mean filament length (1190 m) was in the treatment Sodium bicarbonate followed by Rifampicin (1166 m) and Ornidazole (1160 m). The highest filament length (1205 m) was recorded at 0.8 per cent concentration followed y (1187 m) filament length of Ornidazole at 1.25 per cent concentration. Rifampicin recorded the filament length at par with the control.

The present findings are in conformity with the study of Zargar *et al.* (2001) who found 744.56 m filament length with the use of chemical Codrinal at 0.4 per cent concentration. However, the filament length under the influence of

Sodium bicaronate under the present study has been higher which confirms that the chemicals used in the present study have positive effect on the traits under study.

The discussion of present investigation revealed that pebrine disease in silkworms can be checked to a greater extent by treating the infected silkworms with Sodium bicarbonate at appropriate stage with desired concentration In the present case of sodium bicarbonate at 0.8 per cent concentration has given very good survival of larvae, moths and cocoons with significantly good commercial traits. Thus farmers as and when sense that the silkworms are suffering from pebrine infection, use of Sodium bicarbonate at 0.8 per cent concentration can help them in harvesting a good cocoon crop without any deleterious effect on the economic traits. Besides, the chemical Sodium bicarbonate is easily available and is cost effective.

Chapter 6

SUMMARY AND CONCLUSION

The investigation on the "Management of Pebrine Disease in Bivoltine Silkworms Reared under Temperate Climatic Conditions" was carried out during Spring-2018 at KVK, Ganderbal, SKUAST-Kashmir.

Pebrine caused by Microsporidia is an economically important disease of silkworms as it is highly dreadful and poses a great threat to the silk industry. The disease is transovarially transmitted, thus spreads quickly and leads to the total economic loss of farmers if it remains unchecked. Many attempts have been made by various workers to control this disease but the technology which could address the disease significantly was not available that necessitated to work out the better and suitable option which could address the problem of pebrine disease in silkworms in a smoother and cost effective manner.

The chemicals involved in the present investigation were Sodium bicarbonate, Ornidazole and Rifampicin used at a concentration of (0.6, 0.7 and 0.8%), (0.75, 1.0 and 1.25%) and (2.75, 3.0 and 3.25%), respectively.

All the three chemicals under study at various test concentrations suppressed the growth of perbrine (*Nosema sp*.) and showed a significant control over the disease without any adverse effect on commercial traits. Among the three chemicals Sodium bicarbonate at 0.8 per cent concentration was the most effective in the management of pebrine disease in the silkworm. The other two chemicals i.e; Ornidazole and Rifampicin were statistically at par with each other.

The highest larval survival (93.17%) in 3rd age, (89.22%) in 4th age and (87.39%) in 5th age was observed in case of Sodium bicarbonate at 0.8 per cent concentration followed by Ornidazole and Rifampicin.

Among the three chemicals evaluated in the present study the highest cocoon yield by number (8550) and cocoon weight (19.92 kg) was recorded in the

treatment Sodium bicarbonate at 0.8 per cent concentration, though the two treatments Ornidazole and Rifampicin were next in rank but statistically at par with each other.

The maximum percentage of live cocoons (97.80) was also shown by the treatment Sodium bicarbonate at 0.8 per cent concentration followed by Ornidazole (95.74%) at 1.25 per cent concentration and Rifampicin (94.56%) at 3.25 per cent concentration.

The effectiveness of the treatment Sodium bicarbonate was even proved by the emergence of highest moth percentage (95.93%) at 0.8 per cent concentration. The other two treatments Ornidazole and Rifampicin were also effective but were statistically at par with each other and significantly different than Sodium bicarbonate.

The highest recovery percentage of dfl (96.31%) was recorded by the Sodium bicarbonate at 0.8 per cent concentration followed by Rifampicin (95.55%) at 3.25 per cent concentration and Ornidazole (95.40%) at 1.25 per cent concentration.

The highest single cocoon weight (2.39 g) was also observed in the treatment Sodium bicarbonate at 0.8 per cent concentration followed by Ornidazole at 1.25 per cent concentration recorded (2.37 g) single cocoon weight. However, the results of all the three chemicals were statistically at par with each other.

The maximum single shell weight (0.43 cg) was shown by the treatment Sodium bicarbonate at 0.8 per cent concentration. The two treatments Ornidazole and Rifampicin however, exhibited shell weight (0.36 g) and were at par with each other. Sodium bicarbonate surpassed significantly the other two chemicals.

The maximum shell percentage (19.66) was recorded at 0.8 per cent concentration of treatment Sodium bicarbonate. However, all the three treatments were at par with each other with Ornidazole having 19.41 per cent shell

percentage at 1.25 per cent concentration and Rifampicin 19.14 per cent shell percentage at 3.25 per cent concentration.

The highest silk filament length (1205 m) was recorded at 0.8 per cent concentration of Sodium bicarbonate. Statistically no significant difference was observed among the other two treatments, v.i.z., Ornidazole and Rifampicin.

CONCLUSION

The present study has revealed that all the three chemicals have significant anti-protozoan disease (Pebrine) activity. However, Sodium bicarbonate at 0.8 per cent concentration has been found most effective in the control of pebrine disease of silkworms without any deleterious effect on the performance of commercial cocoon traits like larval duration, weight of ten matured larvae, single cocoon weight, single shell weight, shell percentage and average cocoon filament length. Thus, the management of pebrine disease with the Sodium bicarbonate seems to be farmer friendly, also because of its low cost and easy availability. The other two chemicals namely Ornidazole and Rifampicin have also been found effective against pebrine disease of silkworms, but Sodium bicarbonate (0.8% concentration) surpassed these two in efficacy. Studies also reveal that with the increase in concentration of the chemicals the overall effect on results has also increased. From the studies it is clear that Sodium bicarbonate (0.8 per cent) which is highly cost effective and readily available could be recommended for controlling the dreadful pebrine disease in bivoltine silkworm (*Bombyx mori* L.) under temperate climatic conditions and farmers can also harvest a good cocoon crop with good returns.

LITERATURE CITED

Abe, Y. and Fujiwara, T. 1979. Mode of multiplication of protozoan, Pleistophora sp. Microsporidia–Nosematidae) in the midgut epithelium of the silkworm larvae. *Journal of Seri cultural Science*. Japan **48**: 19-23.

Adeva-Andany. M. M., Carlos. F. F., David. M. B., Elvira. C. C. and Alberto. D. M. 2014. Sodium bicarbonate therapy in patients with metabolic acidiosis. *The Scientific World Journal* p. 13.

Anonymous, 2018. Note on performance of Indian Silk Industry and functioning of Central silk board pp. 23-26.

Baig, M., Samson, M. V., Sharma, S. D., Balavenkata subbaiah, M., Sasidharan, T. O. and Jolly M. S. 1988. Effect of certain disinfectants as surface sterilants against pebrine inn surface contaminated laying. *Sericologia* **28**: 81-87.

Balavenkatasubbaiah, M., Datta, R. K., Nataraju, B. and Iyengar M. N. S. 1994. Efficacy of bleaching powder as disinfectant against the pathogen of silkworm, *Bombyx mori L*. *Indian Journal of Sericulture* **33**(1): 23-26.

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.

Bhat, S. A., Nataraju, B. and Bashir, I. 2012. Efficacy of different benzimidazole derivatives on Microsporidiosis of Lamerin breed of the silkworm, Bombyx mori. L. *Journal of Entomology and Nematology* **4**(2): 12-14.

Boohene C. K., Geden C. J. and Becnd J. J. 2003. E valuation of Remediation Methods for Nosema Disease in *Muscidifurax raptor*. *Environmental Entomology* **32**(5): 1146-1153.

Canning, E. U., Curry, A., Cheney, S. A., Lafranchi-Tristem, N. J., Kawakami, Y., Hatakeyama, Y., Iwano, H. and Ishihara, R. 1999. *Nosema tyriae* and *Nosema sp*., Microsporidian Parasites of Cinnabasr Moth Tyria Jacobaeae. *Journal of Invertebr. Pathology* **74**: 29-38.

Chadrasekharan. J., Kumar. J. J and Sivaprasad. V. 2017. A review on the classification of microsporidia, a 'Taxonomic Nomad'. *Indian Journal of Sericulture* **56**(1-2): 1-9

Chakrabarty, S., Saha, A. K., Bindro, B. B., Manna, B. and Kumar, S. N. 2013. Applied biological research. An improved method for the detection of pebrine (*Nosema bombycis. N.*) spores in silkworm (*Bombyx mori.* L). **15**(2): 91-96.

Chandra, A. K. and Saha-Kudu, 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 carendazium as an anti-microsporidial aget and its influence on the growth and cocoon characters of silkworm, *Bombyx mori. L. Insect Science 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.

Dar, K. A. 2014. Comparative study of mulberry leaf quality ad silkworm rearing performance under different agro-climatic zones of Kashmir and Ladakh. Ph. D Thesis submitted to SKUAST-K pp. 79-80.

Fujiwara, T. 1979. Infectivity and pathogenecity of Nosema bombycis to larvae of the silkworm. *Journal of Seri cultural Science*. Japan **48**: 376-380.

Ganie, N. A., Sahaf, K. A., Munshi, N. A., Zargar, M. A. and Baba, Z. A. 2008. Incidence of protozoan diseases (Pebrine) of silkworm *Bombyx mori.* L. in Kashmir *SKUAST Journal Research* **10**: 134-138.

Goo, Y. K., Terkawai, M. A., Jia, H., Aboge, G. O., Ooka. H., Kim, S., Igarashi, I., Nishikawa, Y. and Xuan, X. 2010. Artesunate a potential drug for treatment of Babesia infection. *Parasitology International* **59**: 481-486.

Griyaghey, U. P. and Kumar, P. 1983. Quantification of Nosema sp. Spores in *Antheraea mylitta* D. Annual report, 1982-83. Central Tasar Research and Training Institute, Ranchi pp. 150-151.

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.

Han, M. S. and Watanabe, H. 1988. Transovarian transmission of two microsporidia in the silkworm, *Bombyx mori.* L and disease occurrence in the progeny population. *Journal of Invertebr. Pathology* **51**: 41-45.

Hatakeyama Y. and Hayasaka, S. 2001. Specific amplification of microsporidia DNA fragments using multiprimer PCR. *Journal of Sericulture Science Japan* **70**: 163-166.

Hatakeyama, Y. and Hayasaka 2003. A new method of pebrime inspection of silkworm eggs using multiprimer PCR *Journal of Sericultural Science, Japan* **82**: 148-151.

Ishihara, R. 1963. Effect of injection of Nosema bombycis (Nageli) on pupal development of the silkworm, *Bombyx mori* L. *Journal of Insect Pathology* **5**: 131-140.

Jolly, M. S. 1986. Pebrine and its control. Bangalore, India: Central Silk Board Publication.

Jolly, M. S., Samson, M. V., Baig, M. M. and Nataraju, B. 1981a. Incidence of silkworm diseases in Karnataka. Annual report 1980-81. Central Sericultural Research and Training Institute, Mysore pp. 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 pp. 113-115.

Kamili, Afifa. S. and Masoodi: M. Amin. 2000. *Principles of Temperate Sericulture*. Kalyani Publishers **20**: 163-169.

Bhat. SA, Ifat .B, Kamili, Afifa. S. 2009. Microsporidiosis of silkworm, Bombyx mori L. (Lepidoptera- Bombycidae). *African Journal of Agricultural Research* .Vol. **4** (13), pp. 1519-1523.

Kishore, S., Baig, M., Natarajan, B., Balvenkatasubbaiah, M. and Datta, R. K. 1994. Cross infectivity of microsporidians isolated from wild Lepidopterous insects to Silkworm, *Bombyx mori*. L. *Indian Journal of Sericulture* **33**(2): 126-130.

Kochar, D. K., Aseri, S., Sharma, B. V., Bumb, R. A., Mehta, R. D. and Purohit, S. K. 2000. The role of rifampicin in the management of Cutaneous Leishmaniasis. *Q. Journal of Med*. **93**: 733-737.

Krishnaswami, S., Narasimhanna, M. N., Suryanarayan, S. K. and Kumararaja, S. 1973. Manual of Sericulture Silkworm rearing Agriculture. *Science Bulletin* **2**: 15.

Krishnaswami, S., Narasimhanna, M. N., Suryanarayana, S. K. and Kumararaj, S. 1978. Sericulture Manual-2 silkworm rearing FAO, Agricultural Sciences Bulletin, Rome **15**(2): 1-24.

Krishnaswami. S., Madhava. R and Suryanarayan. S. K. 1981. *Silk Reeling Manual* **3**: 7-9.

Kumar, K. K. P., Sinha, A. K., Gupta, S. K., Madhu-Sudhan, K. and Prasad, B. C. 2011. Competency in mother moth examination methods of Antheraea mylitta D. I the detection of pebrine spores ad quality evaluation. *International Journal of Zoology Research* **7**: 376-381.

Kurt, O., Giorginkardesler, N., Balcioglu, I. C., Ozbilgin, A. and Ok, U. Z. 2008. A comparison of metronidazole and single dose ornidazole for the treatment of dientamoebiasis. *Clinical Microbiology and Infection* **14**: 6.

Liu, T. P. 1990. Ultra structural changes in Nosema apis within the midgut of honeybees exposed to anti-protozoal drugs in vitro. *Journal of Apicultural Research* **29**(3): 159-164

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

Murakami, A. and Ohtsukhi, Y. 1989. Genetic studies o tropical races of silkworm (*Bombyx mori*) with special refrence to cross breeding strategy

between tropical ad temperate races. *Japan Agriculture Research Quarterly* **23**(1): 37-45.

Nageswara, R. S. and Fujiwara, T. 1994. Studies on mortality rate in multivoltine, bivoltine and multi-voltine hybrid silkworm larvae infected transovarially with *Nosema bombycis*. *Annual report* 1993-94. Central Sericultural Research and Training Institute, Mysore pp. 97-98.

Nataraju, B., Sathyaprasad, K., Manjunath, D. and Aswani Kumar, C. 2005. Silkworm Crop Protection. Bangalore, India: Central Silk Board pp. 1-412

Ozbilgin, A., Ertan, P., Yereli, K. and Onag, A. 2002. Giardiasis treatment in Turkish children with a single dose of Ornidazole. *Infectious Diseases* **34**(12): 918-920.

Peng, L., Christopher, Q. L. and Zisheng, Z. 2015. Cultivation of freshwater green algae Neochloris oleobundans in non-sterile media co-inoculated with protozoa. *The Canadian journal of Chemical Engineering* **94**(3): n/a-n/a.

Prakash, V. N. B. and Dandin, S. B. Factors influencing the adoption of bivoltine sericultural practices in Mandya district of Karnataka. *Indian Journal of Sericulture* **44**(1): 55-58 (2005).

Quadri, S. M. H. and Khatri, R. K. 2005. Strategic management of pebrine in silkworm seed production. *Asian Textile Journal* **14(**1-2): 72-75.

Rajan, R. K. and Himantharaj, M. T. 2005) A Textbook on Silkworm Rearing Technology. *Central Silk Board* pp. 44-50.

Sahaf, K. A. 2002. Incidence of protozoan disease (pebrine) of silkworm, Bombyx mori L. in Jammu and Kashmir. *Journal of Entomology Research* **26**: 305-307.

Saha-Kundu, A. K. and Chandra, A. K. 1978. Studies on the effect of BBenomyl and Mebendazole o perinised silkworm larvae, its control. Annual Report. 1977-78. *Central Sericultural Research Station. Berhampore* (West Bengal) pp. 31-33.

Saha-Kundu, A. K. and Chandra, A. K. 1979. Pebrine disease in silkworms and its control. Annual report 1979-80. *Central Sericultural Research Station. Berhampore* (West Bengal) pp. 31-33.

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

Salim, M. M. and Kandi, E. 1972. Rifampicin in the treatment of cutaneous leishmaniasis. *Journal of Kuwait Med Association* **6**: 159-166.

Samson, M. V., Santha, P. C., Singh, R. N. and Sasidharan, T. O. 1999. A new microsporidian infecting *Bombyx mori* L. *Indian Silk* **37**: 10-12.

Santha, P. C., Singh, R. N., Dutta, R. N. and Singh, R. 2012. Decol-A new general disinfectant effective for controlling secondary pebrine contamination in silkworm crops. *Mun. Entomology Zoology* **7**: 2.

Schmahl, G. and Melhorn, H. 1989. Treatment of fish parasites. Effect of syn. Triazinone (toltrazuril) on developmental stages of Glugea anomala Moniez. (Microsporidia). *European Journal of Parasitology* **24**: 252-259. [Biology Abstract **88**(3): 23571].

Seastian, G. and Elke, G. 2015. Identification of candidate agents active against *N. Ceranae* infection in honey bees: establishment of a medium through put screening assay based on *N. Ceranae* infected culture. *PLOS ONE* **10**: e0 11 7200.

Selva-Kumar, T., Mary, F. C. A., Kishore, S. and Nageswara, R. S. 1993. Incidence of silkworm diseases in sericulture areas of Karnataka. Annual report 1993-94. *Central Sericultural Research and Training Institute*, Mysore pp. 99-100.

Sharma, S. D., Chandrasekharan, K., Nataraju, B., Balavenkatasubbaiah, M., Selvakumar, T., Thiagrajan, V. and Dandin, S. B. 2003. The cross infectivity between a pathogens of silkworm, *Bombyx mori* L. and mulberry leaf roller, Diaphania pulverulentalis (Hampson). *Sericology* **43**: 203-209

Sheetz, R., Goolsby, J. and Poprawksi, T. 1997. Antibiotic treatment of a Nosema sp. (Protozoa: Microsporida) infecting the ovaries of a parasitic Encarsia wasp (Hymenoptera: Aphilindaea). Subtrop. *Plant Science* **49**: 50-52.

Sichtova, M., Vavra, J., Haque, M. A., Canning, E. U. and Robert, G. M. 1993. Evaluation of the antibiotic Sinefungin as an anti microsporidial drug, *Folia Parasitolgica* (Czeck Republic) **40**(2): 85-91.

Singh, R. N., Yadav, P. R. and Singh T. 1992. Containing the pebrine problem in Sericulture. *Indian Silk* **30:** 43-44.

Singh, T. and Saratchandra, B. 2003. Microsporidian disease of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *International Journal of Industrial Entomology* **6**: 1-9.

Singh, T., Bhat, M. M. and Khan, M. A. 2010. Silkworm Egg Science – Principles and Protocols. New Delhi: Daya Publishing House pp. 276

Sprague, V. 1982. Microspora. **In**: *Synopsis and classification of living organism* (Ed. S. P. Parker). New York: McGraw Hill Book Co. pp. 589-594.

Srikanta, H. K. 1987. Studies on Cross infectivity and Viability of *Nosema bombycis N*. (Microsporidia: Nosematidae) M. Sc Thesis University of Agricultural Sciences, Bangalore, India pp. 1-106.

Tayal, M. K. and Chauhan T. P. S. 2017. Silkworm diseases and pests. *Industrial Entomology* pp. 265-289.

Weiser, J. 1969. Immunity of insects to protozoan. **In**: *Insects Immunity to Parasitic Animals* (Eds. C. J. Jackson, R. Herman and I. Singer). New York: Appleton Century Crofts pp. 129-147.

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, Afifa. S., Masoodi, M. A., Munshi, N. A., Tanki, T. N. and Dar, H. U, 2002. Distriution and incidence of Perine disease in various commercial races/ hyrids of silkworm, *Bombyx mori*. L. in Kashmir. *Applied Biological Research* **4**: 39-42.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Prof. Azra N. Kamili during Viva-Voce examination held on 14-02-2020 have been incorporated in the manuscript entitled **"Management of Pebrine Disease in Bivoltine Silkworms Reared under Temperate Climatic Conditions"** submitted by **Ms. Arbia Fatima (Reg. No. 2017-S-52-M).**

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